FORCED DEGRADATION STUDIES OF PYRAZINAMIDE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY AND UHPLC METHOD"

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY
CHENNAI-600032

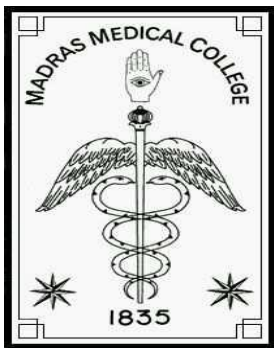
In partial fulfillment of the requirements
for the award of the degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
Reg. No. 261415718

Under the Guidance of
Dr. M.SATHISH M.Pharm., Ph.D.,

Assistant Professor, Department of Pharmaceutical Chemistry



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CERTIFICATE

This is to certify that the dissertation entitled "**FORCED DEGRADATION STUDIES OF PYRAZINAMIDE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY AND UHPLC METHOD** '' is submitted by the candidate bearing the Register No. **261415718** in partial fulfillment of the requirements for the award of degree in **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** by The Tamil Nadu Dr.M.G.R Medical University ,Chennai, is a bonafide work done by her during the academic year **2015-2016** at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical college,Chennai-03 .**

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ACKNOWLEDGEMENTS

First and foremost I will thank Almighty God, the compassionate, the almighty Merciful, who kindly helped me to complete my thesis.

I am grateful to express my sincere thanks to **Dr.VIMALA, M.D**., Dean Madras Medical College for giving an opportunity to carry on with my project work.

I would like to express my grateful gratitude and sincere appreciation to our valuable advise, supervision encouragement and kindness to our respected, **Dr.A.JERAD SURESH, M.Pharm., PhD., M.B.A.,** Principal, Professor and Head, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College for his whole hearted support in rendering me all the facilities required for my project work.

It is my pleasure to express my deep and heartfelt sense of gratitude to my guide **Dr.M.SATHISH, M.Pharm., Ph.D**., Assistant Professor in Pharmacy, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College for him active guidance, advice, help, support and encouragement carrying out my project work.

It is my utmost duty and privilege to express my heartfelt gratitude to **Dr. (Mrs.) V.NIRAIMATHI M.Pharm. PhD, Assistant Professor in Pharmacy,** Department of Pharmaceutical Chemistry, College of Pharmacy,

I thank **to Mrs. Dr. R. Priyadarshini M.Pharm. Ph.D. Mrs. Dr.P.G.Sunitha, M.Pharm. Ph.D., Mrs.T.Sarawasthy M.Pharm. (Ph.D.),** Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College for their timely help and cooperation towards completing this project.

I thank to the Director of Medical Education, Directorate of education, Kilpauk, Chennai- 600010 for their constant support to complete my project work.

My heartfelt thanks to my loveable brother for his motivation and also thank my Sister and **G.sudha, G.Kowsi, K.Kannan, for this support.**

I thank my team leader **Mr. Joseph Raja B.Pharm**., and his family, Synthiya Research Lab Pvt. Ltd., and also thank to **Mr.Rajasekar M.Sc.,** and his family for kind support throughout my project work.

I thank to my friends **A. Alagesan, M.Pharm, G.Maheshkumar M.Pharm, V.S saithanisha G.Viswa M.E., S.Veera B.E for the constant support.**

I thank **Mr.D.Sivakumar, Mr.Baskar Lab supervisor Mrs.Maheshwari, M.Murgeswari** for this support, Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College.

I Thank my classmates **M.Neelakandan, K.Madhesh, R.Pandiyan, R.Ravikumar ,N.Ramya, R.Kalaisevi, B.Karunya, S.Mala ,S.priya and all my juniors.**

I am so happy to express my sincere love and sense of gratitude to my beloved **Father and Mother** and Family members for their excellent cooperation and support extended throughout the project.

CONTENTS

LIST OF ABBREVIATIONS

ABBREVIATION	EXPANSION
%	Percentage
µg	Microgram
µl	Microliter
Abs	Absorbance
API	Active Pharmaceutical Ingredient
Avg.	Average
Cm	Centimeter
Dil.	Dilution
G	Gram
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric Acid
UHPLC	Ultra High Performance Liquid Chromatography
IR	Infrared
KBr	Potassium Bromide
Mg	Milligrams
Mins	Minutes
Ml	Milliliter
NaOH	Sodium hydroxide
NM	Nanometer
°	Celsius
Rf	Retention Factor
Sam	Sample
Std	Standard
UV	Ultra Violet
Vol	Volume
Wt.	Weight
λ	Lambda

INTRODUCTION

PHARMACEUTICAL ANALYSIS:

Pharmaceutical analysis may be defined as a process or a sequence of process to identify and or quantify a substance or drug, the components of a Pharmaceutical solution or mixture or the determination of the structure of chemical compounds used in the formulation of Pharmaceutical product.[1]

CLASSIFICATION:

Pharmaceutical analysis can be classified in the various ways

All pharmaceutical analysis process can be categorized into two groups:

1. Qualitative (Identification)

2. Quantitative (Estimation)

1) ***Qualitative analysis:*** the analysis of a substance in order to ascertain the nature of its chemical constituents. It is mainly used in the determination of assay of samples.

2) ***Quantitative analysis:*** Chemical analysis designed to determine the amount or proportion of the components of a substance. It is mainly used in the degradation studies to determine the amount of drug and degraded products present in the sample.[2]

Various types of qualitative analysis:

1) Chemical Methods
   a. Volumetric or Titrimetric Methods
   b. Gravimetric Methods
   c. Gasometric Analysis
2) Electrical Methods
3) Instrument Methods
4) Biological and Microbiological.

DEGRADATION STUDIES:

Forced degradation studies are also known as stress testing, stress studies, stress decomposition studies, forced decomposition studies, etc. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used. But these guidelines are very general in conduct of forced degradation and do not provide details about the practical approach towards stress testing. [3]

Although forced degradation studies area regulatory requirement and scientific necessity during drug development, it is not considered as a requirement for formal stability program. It has become mandatory to perform stability studies of new drug moiety before filing in registration dossier. The FDA and ICH guidance's state the requirement of stability testing data to understand how the quality of a drug substance and drug product changes with time under the influence of various environmental factors. The stability studies include long term studies (12months) and accelerated stability studies (6months).But intermediate studies (6months) can be performed at conditions milder than that used in accelerated studies. So the study of degradation products like separation, identification and quantitation would take even more time.

2. OBJECTIVE OF FORCED DEGRADATION STUDIES:

Forced degradation studies are carried out to achieve the following purposes:

1) To establish degradation pathways of drug substances and drug products.

2) To differentiate degradation products that is related to drug products from those that are generated from non-drug product in a formulation.

3) To elucidate the structure of degradation products,

4) To determine the intrinsic stability of a drug substance in formulation.

5) To reveal the degradation mechanisms such as hydrolysis, oxidation, thermolysis or photolysis of the drug substance and drug product

6) To establish stability indicating nature of a developed method.

7) To understand the chemical properties of drug molecules.

8) To generate more stable formulations.

9) To produce a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.

10) To solve stability-related problems[4, 5]

Forced degradation performed:

It is very important to know when to perform forced degradation studies for the development of new drug substance and new drug product. FDA guidance states that stress testing should be performed in phase III of regulatory submission process. Stress studies should be done in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to determine the stability of the drug substance.

These stress studies are conducted on a single batch. The results should be summarized and submitted in an annual report. However, starting stress testing early in preclinical phase or phase I of clinical trials is highly encouraged and should be conducted on drug substance to obtain sufficient time for identifying degradation products and structure elucidation as well as optimizing the stress conditions. An early stress study also gives timely recommendations for making improvements in the manufacturing process and proper selection of stability-indicating analytical procedures.[6, 7]

Degradation of drug substances between 5% and 20% has been accepted as reasonable for validation of chromatographic assays[8]. Some

pharmaceutical scientists think 10% degradation is optimal for use in Analytical validation for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common[9]. Others suggested that drug substance spiked with a mixture of known degradation products can be used to challenge the methods employed for monitoring stability of drug product. No such limits for physiochemical changes, loss of activity or degradation during shelf life have been established for individual types or groups of biological products [10, 11].

It is not necessary that forced degradation would result in a degradation product. The study can be terminated if no degradation is seen after drug substance or drug product has been exposed to stress conditions than those conditions mentioned in an accelerated stability protocol [12]. This is indicative of the stability of the molecule under test. Over-stressing a sample may lead to the formation of a secondary degradation product that would not be seen in formal shelf-life stability studies and under-stressing may not generate sufficient degradation products [13]. Protocols for generation of product-related degradation may differ for drug substance and drug product due to differences in matrices and concentrations. It is recommended that maximum of 14 days for stress testing in solution (a maximum of 24h for oxidative tests) to provide stressed samples for methods development.[14]

IMPURITY PROFILING

The definition of the impurity profile of a new drug material as given in the guidelines of ICH is "A description of the identified and unidentified impurities, present in a new drug substance". As for impurity profiling, it is the common name attributed to analytical activities performed with the aim of detecting, identifying or elucidating the structure and quantitatively determining and inorganic impurities as well as residual solvents in bulk and pharmaceutical formulation.

If the impurities of a drug material (the difference between 100% and the 98-99% mentioned above) are physiologically highly active (toxic) materials, in principle they could contribute to the side effect profile of the

drug .since the impurity profile of a drug material depend on the synthesis route and other factors, this could make the side –effect profile irreproducible, adversely influencing the safety of the drug therapy. By estimating the impurity of a drug material and setting strict limits for the impurities, this danger can be minimized.[15]

1.1. TYPES OF STABILITY STUDIES

There are three types of stability studies, which give an idea about the stability of drugs.

A. Long term stability studies

B. Accelerated stability studies and

C. Intermediate testing:

Long term stability testing (Real Time Testing):

The length of the studies and the storage conditions should be sufficient to cover storage, shipment and subsequent use Temperature sensitive drug substances should be stored under an alternative lower temperature condition, which will then become the designated long term testing storage temperature. Storage for long term testing- 25°C ± 2°C/ 60 % RH ± 5 % RH, minimum time Period for submission 12 months .

Accelerated stability testing:

To introduce new products into the market or for making material changes in the process, formula, or container closure system of existing products one cannot wait until all the needed stability data at room temperature are generated. These studies are designed to increase the rate of chemical degradation or physical change of an active drug substance or drug product by using exaggerated storage conditions as part of the formal, definitive storage program. Results from accelerated testing studies are not always indicative of physical change. Storage condition for accelerated testing 40°C ± 2°C/ 75 % RH ± 5 % RH, minimum time period for submission 6 months

Intermediate testing:

Intermediate studies are conducted at 30°C/65% RH and designed to moderately increase the rate of chemical degradation or physical changes for a drug substance intended to be stored long term at 25°C Generally these studies are conducted when the accelerated studies for General Case (40°C/75 % RH) failed to meet the acceptance criteria.[16]

Table-1 Conditions used for force degradation studies [17]

Solid state

Stress	Condition	Period of time
Heat	60°C	1month
Humidity	75°C	1 month
Photo stability	Exposed And Non Exposed Sample (Control)	Follow ICH requirements

Solution state

Stress		Condition	Period of time
hydrolysis	**Acid**	0.1-1 Mol L-1 HCl	Up to weeks and 60ºC
	Alkali	0.1-1 Mol L-1 NaOH	Up to weeks and 60ºC
Oxidation		3% (v/v)H_2O_2	Up to 24HOURS
Photo stability		Exposed and non-exposed sample (Control)	Follow ICH requirements
Heat		60ºC	Up to 1 month

CHARACTERISATION OF DEGRADADED PRODUCTS

The characters of the degraded fragments were studied by both spectrophotometric methods and chromatographic techniques. The degraded products were analyzed by following methods.

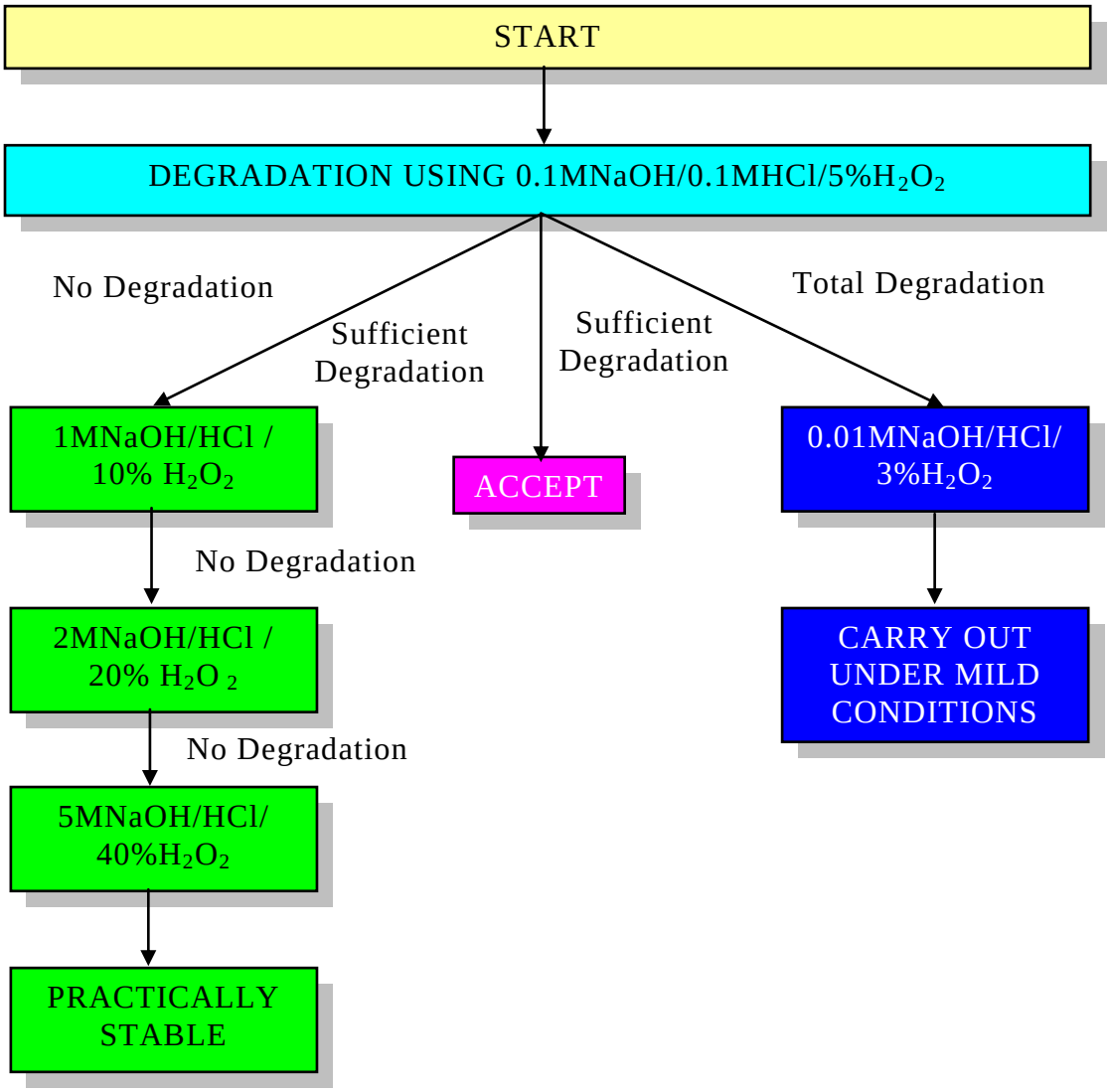
UV SPECTROSCOPY

INFRA RED SPCTROSCOPY

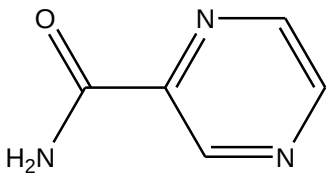
UHPLC

These methods were used to determine the assay and the amount of sample present in the degraded products and also used in identification of impurities

SCHEME OF FORCED DEGRADATION

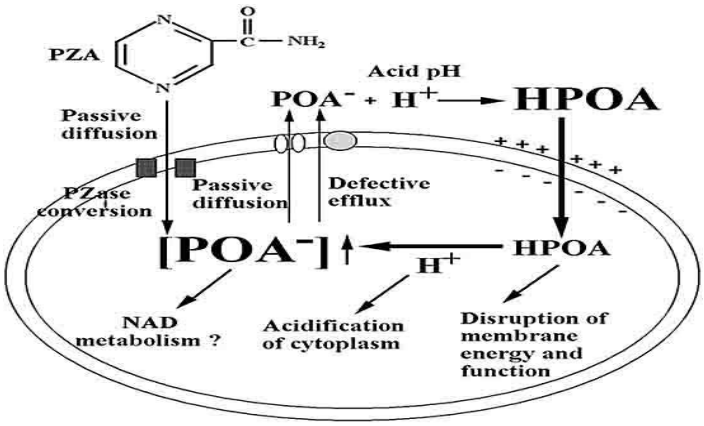


DRUG PROFILE

DRUG NAME	PYRAZINAMIDE
BRAND NAME	Pyzina-1000mg
IUPAC NAME	Pyrazine-2-carboxamide
MOLECULAR NAME	$C_5H_5N_3O$
MOLECULAR WEIGHT	123.1g/mol.
CAS NUMBER	98-96-4
CHEMICAL STRUCTURE	
BASIC MOIETY	Pyrazine nucleus
CATEGORY	First line drug, Antitubercular agent
DESCRIPTION	white or almost white crystalline powder odorless or almost Odorless
SOLUBILITY	It is soluble in water and in chloroform; slightly soluble in ethanol 95% and slightly soluble in ether. [18]

MECHANISM OF ACTION

Pyrazinamide is a prodrug that stops the growth of mycobacterium tuberculosis. Pyrazinamide diffuses into the granuloma of mycobacterium tuberculosis, where the enzyme Pyrazinamidase converts Pyrazinamide to the active form pyrazinoic acid under acidic conditions, the pyrazinoic acid that slowly leaks out converts to the protonated conjugate acid which is thought to diffuse easily back into the bacilli and accumulate the net effects that more pyrazinoic acid accumulates inside the bacillus at acid pH than neutral pH.[19]



Pyrazinoic acid was thought to inhibit the enzyme fatty acid synthase (FAS) in, which is required by the bacterium to synthesise fatty acids although this has been discounted.

DRUG INTERACTIONS: Pyrazinamide Interactions gout, hepatotoxicity, liver diseases renal dysfunction, diabetes mellitus, hemodialysis

PREGNANCY: Pyrazinamide only recommended during pregnancy when benefit outweighs risk.

NURSING MOTHERS: Pyrazinamide is excreted into human milk in small amounts. The manufacturer recommends that caution be used when administering pyrazinamide to nursing women.

SIDE EFFECTS: Pain in large and small joints, loss of appetite, pain and swelling of joints, especially big toe ankle and knee, unusual tiredness or weakness yellow eye or skin rarely producing itching, skin rash.

DOSING: Recommended drugs for the initial treatment of tuberculosis in children and adults daily dose=15mgto30mg/kg po (per orally),A single 1000mg dose may used for tuberculosis,3 grams per day should not exceed, 2gm per day when given as daily regimen

It is administered orally.

INDICATIONS:

Pyrazinamide is used to treat tuberculosis (TB).It is an antibiotic and works by stopping the growth of bacteria. This antibiotic treats only bacterial infections[20].

REVIEW OF LITERATURE

1) **Abdel *et al*.,(2015)** reported a development and validation of a quantitative and a stability –indicating densitometric method for sensitive estimation of some active TB drugs, As the stationary phase and the eluent comprised two mobile phase were selected. Chloroform-ethanol isopropanol (4.5:5.25:5.25v/v/v)and ethanol –chloroform-ammonia (33%) 7:7:0.5 v/v/v) densitometric analysis was achieved at λmax270nm for rifampicin isoniazid and pyrazinamide stress was carried by under acid base oxidation and photolytic degradation.[21]

2) **Drashti Desai, Megha Shah. (2015)** a review article on validated analytical methods developed on Antitubercular Drug, Rifampicin. The clinical and pharmaceutical analysis of this drug requires effective analytical procedures for quality control and pharmacodynamics and pharmacokinetic studies as well as stability study. An extensive survey of the literature published in various analytical and pharmaceutical chemistry related journals has been conducted and the instrumental analytical methods which were developed and used for determination of Rifampicin as single or combination with other drugs in bulk drugs, formulations and biological fluids have been reviewed.[22]

3) **Blessy M *et al*., (2014)** a review article on development of forced degradation and stability indicating of drugs. Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. It is required to demonstrate specificity of stability indicating methods and also provides an insight into degradation pathways and degradation products of the drug substance and helps in elucidation of the structure of the degradation products. Forced degradation studies show the chemical behavior of the molecule which in turn helps in the development of formulation and package.[23]

4) **Nerdy *et al.*, (2014)** evaluated high performance liquid chromatography mass spectrometry method to analyze a mixture of rifampicin, isoniazid and pyrazinamide. C18 column as stationary phase and mass spectrometry as a detector in selected ion monitoring mode.[24]

5) **Paolo miotto *et al.*,(2014)** reported a Mycobacterium tuberculosis pyrazinamide resistance determinants: Multicenter study, Pyrazinamide is a prodrug that is converted to pyrazinoic acid by the enzyme Pyrazinamide, encoded by the pncAgene in mycobacterium tuberculosis.[25]

6) **M.S. Charade *et al.*, (2013)** a review article on forced degradation studies of new drug substances and drug products are important to help develop and demonstrate specificity of stability-indicating methods and to determine the degradation pathways and degradation products of the active ingredients. Conventional methods (e.g. Column chromatography) or hyphenated techniques (e.g.LC MS, LCN MR)can be used to identification and characterization of the degradation products.[26]

7) ***Abdel Maaboud et al.*, (2013)** developed a simple and sensitive method for determination of ethambutol (EMB), isoniazid (INH), pyrazinamide (PZA) and rifampicin (RIF) in pure and pharmaceutical dosage forms. The method is based on measuring the quenching effect of studied drugs on the fluorescence intensity of NBS-phenothiazine oxidation product (NBS-Phz) in a buffered medium (pH 7, λ_{ex} 271 and λ_{em} 375 nm). Different factors affecting the reaction were studied and optimized. Under the optimized conditions, linear relationships with good correlation coefficients (0.9995-0.9999) were obtained. The limits of detection were 0.139, 6.39×10^{-3}, 0.029 and 0.180 µg ml^{-1} for EMB, INH, PZA and RIF respectively.[27]

8) **Kishore Kumar Hotha *et al.*, (2013)** a review article to give a detailed description of the forced degradation studies as per the regulatory guidelines that are associated with various regulatory agencies. This

article summarizes the collective views of industry practices on the topic of forced degradation studies. The article includes an overview of existing guidance's and literature for best practices.[28]

9) **Ganga Prasad chenna *et al*., (2011)** reported a simple, precise, specific and accurate Reverse phase HPLC method has been developed for the determination of Pyrazinamide in bulk and pharmaceutical dosage forms. C8 (4.6 x 250mm, 3.5 µm) column with Phosphate buffer (pH 4.4): Methanol 80:20 (v/v) as a mobile phase at a flow rate of 1 ml/ min. Detection was performed at 269 nm. The retention time of Pyrazinamide was found to be 3.62min. By Adoption of this procedure Pyrazinamide (PYZ) is eluted completely.[29]

10) 10. **Ganga Prasad chenna *et al*., (2011)** reported, two simple, accurate and precise spectrophotometric methods have been developed for Pyrazinamide in bulk and pharmaceutical dosage form. Pyrazinamide has absorbance maxima at 269nm.Method A is area under curve (AUC) method, which involves the calculation of integrated value of absorbance with respect to wavelength between 264-274nm. Method B involves the derivatisation of the primary absorption spectra to second order and by the examination of the second derivative spectra of Pyrazinamide, 270 nm (λ) was selected as working wavelength.[30]

11) **Saranjit Singh *et al*., (2010)** reported the extent of degradation of rifampicin, isoniazid and pyrazinamide from prepared mixture and marketed preparation containing single ,two,three,and four, drugs under stomach conditions degradation studies carried out in 0.1M HCL at 37 ºC for 50mins. A comparative study in stimulated gastric fluid was also done. Under both conditions rifampicin was decomposed by (17.8-24.4%), isoniazid to a lesser extent (3.2-4.7%) and pyrazinamide was stable.[31]

12) **S.K.Dhal *et al.* (2009)** reported a new reversed –phase HPLC method for the simultaneous analysis of pyridoxine Hcl, Isoniazid, Pyrazinamide, and Rifampicin in a tablet formulation has been

developed. This method is accurate, reproducible, repeatable, linear, precise, selective, and thus reliable. The run time was relatively short, i.e.20min.[32]

13) **M. Y. Khuhawar *et al.*, (2005)** reported a simple HPLC method for determination INH, PZA, and IM in pharmaceutical preparations. Pre-column derivatization with MFA is used for INH. Isocratic elution from a reversed-phase ODS column results in ng per injection detection limits. Analysis of the drugs resulted in CV of 0.54–0.74% with relative deviation up to 4.8%. [33]

14) **John E.*et al.*, (2000)** investigated the measurement of pyrazinamide in human plasma, bronchoalveolar lavage and alveolar cells by reversed phase column chromatography.[34]

15) **A.Brouard *et al.*, (1985) r**eported a gas chromatographic-mass spectrometric technique and published a high-performance liquid chromatographic (HPLC) technique that had a very long and difficult extraction. The present method involves reversed-phase HPLC with UV detection of pyrazinamide.[35]

AIM AND OBJECTIVE

The aim of the study is to develop simple, novel methods for the forced degradation of Pyrazinamide in Bulk and Formulation. The quantification of Pyrazinamide have been reported and review of literature indicated that no degradation studies have been reported for pharmaceutical formulation till date

The objective of the present work is develop various analytical methods such as

- UV -SPECTROPHOTOMETRY
- INFRA RED SPECTROPHOTOMETRY
- UHPLC METHODS

The study includes the following:

- To perform the acid and alkali hydrolysis, oxidative degradation, thermal and Photolytic degradation.
- Quantification of degraded product by UV and UHPLC.
- Comparing the results of bulk and sample with standard.
- Identification of changes in functional group present in the degraded samples by IR

MATERIALS AND METHODS

DRUG SAMPLES AND STUDY PRODUCTS

Pyrazinamide Standard was obtained from Micro Labs in Hosur

TEST PRODUCT

Pyrazinamide tablets (Pyzina -1000mg) were purchased from medical shop.

CHEMICALS AND SOLVENTS USED FOR DEGRADATION

Hydrochloric Acid	Merck, AR Grade, Mumbai, India.
Hydrogen Peroxide (5%W/V)	Merck, AR Grade ,Mumbai ,India
Sodium hydroxide	Merck, AR Grade ,Mumbai ,India
Chloroform	Merck, AR Grade ,Mumbai ,India
Ethanol	Merck, AR Grade ,Mumbai ,India
Methanol	Merck, HPLC Grade ,Mumbai ,India
Acetonitrile	Merck, HPLC Grade ,Mumbai ,India
Water	Double Distilled

INSTRUMENTS USED:

Shimadzu V-630 UV Spectrophotometer	Double beam, UV Probe 2.31
Rankem-IR, KBr Press	Cary 630 FTIR
Agilent UHPLC	Infinity 1220 LC, UV Detector
Electronic Balance	Wensar, PGB-200

FORCED DEGRADATION

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than

accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule.[36]

ANALYTICAL PROCEDURES

Degraded samples were analyzed using spectrophotometric methods like UV, IR and chromatographic methods like UHPLC.The assays of degraded samples were carried out by UV and UHPLC and the changes in the functional group of drug were detected by IR spectroscopy.

HYDROLYTIC DEGRADATION

Hydrolysis usually means the cleavage of chemical bond by the addition of water. The main source of impurities in the formulation is for hydrolytic degradation. Water either as a solvent or a moisture in the air comes in contact with pharmaceutical dosage form is responsible for degradation of most of the drugs. In hydrolytic study under acid and basic condition, pyrazinoic acid is cleaved from pyrazinamide in 0.1M NaOH and 0.1M HCl are employed for generating acidic and basic stress samples respectively. If sufficient degradation is not observed, higher strength are employed to induce degradation (1M NaOH/1M HCl)

OXIDATIVE DEGRADATION

The increase in oxidation state of an atom through a chemical reaction is known as an Oxidation. Most of the drug undergoes auto oxidation because of oxygen in air. Therefore it is an important degradation pathway of many drugs. Auto oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions in a drug substance act as initiator for auto oxidation. Hydrogen peroxide is a common oxidant to produce oxidative degradation products which may arise as minor impurities during long term stability studies. Hydrogen peroxide seems to be much more popular for the purpose than any other oxidizing agent. The strength of H_2O_2 used varies from 1% to 30%.in some drugs extensive degradation is seen when exposed to 3% H_2O_2 for very short time periods at room temperature in other cases, exposure to high concentration of H_2O_2 even under extreme conditions does not cause any significant degradation.

The behavior is on expected lines, as some drugs are in fact or disable, while there are others that are not. The latter are not expected to show any change even in the presence of high dose of oxidizing agents.

THERMAL DEGRADATION

Temperature also plays a role in degradation of drugs. High temperature leads to increase in degradation of drugs. Most of the drugs are sensitive to temperature. Thermal degradation can cause reactions like pyrolysis, hydrolysis, decarboxylation isomerization rearrangement and polymerization

PHOTOLYTIC DEGRADATION

Exposure of sun light to drug is called photolytic degradation. The rate of degradation is directly proportional to the intensity; quantity of sun light absorbed by the drug. It is carried out by exposing the drug substance and drug product to a sun light. (ICH Guidelines) [37]

UV SPECTROSCOPY

UV refers to absorption spectroscopy, spectroscopy in the ultra violet spectral region .this means it uses light in the visible and adjacent (near-UV and near –infrared (NIR)) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. molecules containing π-electrons or non-bonding electron (η-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electron the longer the wavelength of light it can absorb.

In degradation studies UV plays one of the roles in detection of impurities assay values of drug products can be calculated using spectroscopy. In ultra violet region every molecule will give its own absorption at particular wavelength this is called λ max. Every compound has unique λ max and absorption. Absorption values vary only with the concentration of the compound. Degraded samples spectrum can be compared with standard spectrum to identify the degradation .After degradation the

absorption and the λmax of the samples can vary from the standard. This shows the degradation of samples[38].

Materials and Methods

All absorption spectrum were measured by Shimadzu V-630 UV Spectrophotomer with 1cm matched quartz cells.

Reagents used

0.1M Sodium hydroxide

0.1M Hydrochloric acid

5% Hydrogen peroxide

Acetonitrile

Distilled water

PREPARATION OF REAGENTS

Preparation of 0.1M Sodium hydroxide

4 gm of Sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000ml

Preparation of 0.1M Hydrochloric acid

8.33ml of concentrated hydrochloric acid was measured and diluted with distilled water to 1000ml

Preparation of 5% Hydrogen peroxide:

50ml of Hydrogen peroxide was diluted with distilled water and the volume made up to1000ml

Table-2 Intraday scheme UV –study of Bulk and Formulation

Degradation Type	Experimental Conditions	Storage Conditions	Sampling Time
	Control sample	Room temperature	30,60,90 mins
Hydrolysis	0.1M NaOH	Room temperature	30,60,90mins
	0.1M HCl	Room temperature	30,60,90mins
	Acid control	Room temperature	30,60,90mins
	Base control	Room temperature	30,60,90mins
Oxidation	5% H_2O_2(control)	Room temperature	30,60,90mins
	5% H_2O_2	Room temperature	30,60,90mins

Table-3 Inter day scheme UV –study of Bulk and Formulation

Degradation Type	Experimental conditions	Storage conditions	Sampling time
	Control sample	Room temperature	1,3,5 days
Hydrolysis	0.1M NaOH	Room temperature	1,3,5 days
	0.1M HCl	Room temperature	1,3,5 days
	Acid control	Room temperature	1,3,5 days
	Base control	Room temperature	1,3,5 days
Oxidation	5% H_2O_2 (control)	Room temperature	1,3,5 days
	5% H_2O_2		1,3,5 days
Thermal	Heating chamber	50ºc	1,3,5 days
Photolytic		Sunlight	1,3,5 days

INTRADAY STUDY OF HYDROLYTIC DEGRADATION USING 0.1M NaOH

Standard Preparation

100mg Pyrazinamide was transferred to volumetric flask and dissolved in 100ml of distilled water to achieve a concentration of 1mg/ml. The solution was kept at room temperature. An aliquot solution was then diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.

System suitability solution: Transfer of 1ml hydrochloric acid to volumetric flask, dilute with standard preparation to volume mix. Keep the solution on a boiling water bath for 5 minutes and cool. (1:4)

Bulk preparation (Stress)

100mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved 0.1M NaOH to produce 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 60min and 90min time interval.

Sample preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask, dissolved in 0.1 M NaOH to achieve a concentration of 1mg /ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 60mins and 90mins time interval.

Blank preparation

100ml of 0.1M NaOH solution was taken in a 100ml volumetric flask, the solution was kept at room temperature, after 30 mins an aliquot solution

was diluted with distilled water to get final concentration. This is used as a blank. The procedure was repeated thrice .after the stipulated time. The absorption of the resulting solution showed maximum 270 nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

The percentage content of bulk was determined by following formula

Percentage content =

$$\frac{\text{Sample absorbance x Standard weight x Dilution factor x Purity of Standard x100}}{\text{Standard absorbance x sample weight x100}}$$

The amount of present was determined by following formula =

Amount content=

$$\frac{\text{Sample absorbancex Standard weight x Dilution factor xPurity of Standard x Avg.wt tablets}}{\text{Standard absorbance x sample weight x100}}$$

Percentage content of pyrazinamide was determined by following formula

$$\text{Percentage content} = \frac{\text{Amount present}}{\text{Label claim}}$$

HYDROLYTIC DEGRADATION USING 0.1 M HCl

Bulk preparation (stress)

100mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved 0.1M HCl to achieve a concentration of 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of 10 µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270 nm. The same procedure was repeated for 60 min and 90mins time interval.

Sample preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask, dissolved in 0.1M HCl to achieve a concentration of 1mg / ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 60mins and 90mins time interval.

Blank preparation

100 ml of 0.1M HCl solution was taken in a 100ml volumetric flask, the solution was kept at room temperature, after 30 min an aliquot solution was diluted with distilled water to get final concentration; this is used as a blank. The procedure was repeated thrice, after the stipulated time. The absorption of the resulting solution showed maximum 270nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

OXIDATIVE DEGRADATION USING 5%H_2O_2

Bulk preparation (stress)

100mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved to 5% H_2O_2 achieves a concentration of 1mg/ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270 nm same procedure was repeated for 60 mins and 90mins time interval.

Sample preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask, dissolved 5% H_2O_2 to achieve a concentration of 1mg /ml. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get final concentration of

10µg/ml .The solution was scanned in the UV region and the maximum absorbance was recorded at 270 nm. The same procedure was repeated for 60mins and 90mins time interval.

Blank preparation

100ml of 5% H_2O_2 solution was taken in a 100ml volumetric flask. The solution was kept at room temperature. After 30mins an aliquot solution was diluted with distilled water to get final concentration. This is used as a blank. The procedure was repeated thrice .after the stipulated time. The absorption of the resulting solution showed maximum 270 nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

INTER DAY FORCED HYDROLYTIC DEGRADATION USING 0.1 M NaOH

Bulk Preparation (stress)

100mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved 0.1M NaOH to achieve a concentration of 1mg/ml .The solution was kept at room temperature. Then the next day (1^{st} day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 3^{rd} day and 5^{th} day time interval. The obtained spectrum is compared with standard spectrum.

Sample Preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask., dissolved 0.1M NaOH to achieve a concentration of 1mg/ml .The solution was kept at room temperature. Then the next day (1^{st} day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 3^{rd} day and 5^{th} day time interval. The obtained spectrum is compared with standard spectrum.

Blank preparation

100ml of 0.1M NaOH solutions was taken in a 100ml volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice .After the stipulated time. The absorption of the resulting solution showed maximum 270nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

HYDROLYTIC DEGRADATION USING 0.1M HCl

Bulk Preparation (stress)

100mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved 0.1M Hydrochloric acid to achieve a concentration of 1mg/ml .The solution was kept at room temperature. Then the next day (1st day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 3rd day and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample Preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask, dissolved 0.1M Hydrochloric acid to achieve a concentration of 1mg/ml .The solution was kept at room temperature. Then the next day (1st day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm.The same procedure was repeated for 3rd day and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Blank preparation

100ml of 0.1M HCl solutions was taken in a 100ml volumetric flask. The solution was kept at room temperature the next day; an aliquot solution was diluted with distilled water to get final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice .After the stipulated time. The absorption of the resulting solution showed maximum 270nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

OXIDATIVE DEGRADATION USING 5% H_2O_2

Bulk preparation (stress)

100 mg of Pyrazinamide bulk was weighed and transferred to volumetric flask, dissolved to 5% H_2O_2 achieve a concentration of 1mg/ml .The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270 nm same procedure was repeated for 3rd and 5th day time interval.

Sample preparation (stress)

100mg equivalent of Pyrazinamide tablets were crushed weighed and transferred to volumetric flask, dissolved 5% H_2O_2 to achieve a concentration of 1mg /ml. The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The solution was scanned in the UV region and the maximum absorbance was recorded at 270nm. The same procedure was repeated for 3rdand 5thday time interval.

Blank preparation

100ml of 5% H_2O_2 solution was taken in a 100ml volumetric flask, the solution was kept at room temperature. The next day an aliquot solution was diluted with distilled water to get final concentration.

The procedure was repeated thrice .after the stipulated time. The absorption of the resulting solution showed maximum 270nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

THERMAL DEGRADATION

Bulk Preparation (stress)

1gm of Pyrazinamide bulk was weighed and transferred to a Petri dish. This Petri dish was placed in hot air oven at the temperature of 50ºC.The next day 100mg Pyrazinamide bulk was weighed from a Petri dish and transferred to 100ml volumetric flask. It was dissolved in water and the volume made up to 100ml .An aliquot solution was diluted with distilled water to get final concentration of 10µg/ ml. The same procedure was repeated for 3rd and 5th day.

Sample Preparation (Stress)

1gm of Pyrazinamide tablets were weighed and transferred to a Petri dish. This Petri dish was placed in hot air oven at the temperature of 50ºC.The next day 100mg equivalent of Pyrazinamide powder was weighed from a Petri dish and transferred to 100ml volumetric flask. It was dissolved in water and the volume made up to 100ml .An aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The same procedure was repeated for 3rd and 5th day.

Blank Preparation

Distilled water was used a blank

PHOTOLYTIC DEGRADATION USING SUNLIGHT

Bulk preparation (stress)

1gm of Pyrazinamide bulk was weighed and transferred to a Petri dish. This Petri dish was placed under sunlight. The next day 100mg of Pyrazinamide bulk was weighed from a Petri dish and transferred to 100ml volumetric flask. It was dissolved in distilled water and the volume made up

to 100ml .An aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The same procedure was repeated for 3rd and 5th day.

Sample Preparation (Stress)

1gm of Pyrazinamide tablets were weighed and transferred to a Petri dish. This Petri dish was placed under a sunlight .The next day 100mg equivalent of Pyrazinamide was weighed from a Petri dish and transferred to 100ml volumetric flask. It was dissolved in distilled water and the volume made up to 100ml .An aliquot solution was diluted with distilled water to get final concentration of 10µg/ml. The same procedure was repeated for 3rd and 5th day.

Blank Preparation

Distilled water was used a blank

INFRA RED SPECTROSCOPY

Infrared spectroscopy is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, which is light with a longer wavelength and lower frequency than visible light .The infrared portion of the electromagnetic spectrum is usually divided into three region; the near -,mid –and far-infra red, named for their relation to the visible spectrum. The IR region is mainly divided into two types

Finger print region (1500-400cm^{-1})

This will vary for every compound .In degradation studies this region can show a complete degradation of a sample otherwise no changes in the finger print region.

Functional group region (4000-1500cm^{-1})

This will give the peaks based on the functional group present in the compound.

In degradation studies it can shows the changes in functional groups. Standard can be compared with sample to detect the changes after the degradation[39].

IR spectroscopy is mainly used in thermal and photolytic degradation studies.

Apparatus-Agilent Technologies

All spectral measurement was made on (Rankem -IR) (Model No. Cary 630 FTIR KBr Press)

Interday scheme of IR-study for bulk and formulation (Table-4)

Degradation type	Material (solid)	Storage condition	Sampling
Normal	Bulk	Room temperature	1,3,5days
	Sample	Room temperature	1,3,5days
Photolysis	Bulk	Sunlight	1,3,5days
	Sample	Sunlight	1,3,5days
Thermal (Heating Chamber)	Bulk	50ºC	1,3,5days
	Sample	50ºC	1,3,5days

INTERDAY STUDY OF PYRAZINAMIDE BY IR

General Procedure

Pyrazinamide tablets were weighed and transferred into Petri dish. The first one was kept at room temperature, the second one was kept at chamber at 50ºC and the third one was kept at sunlight .This was referred as 0 day. The bulk drug was weighed and transferred into 3different Petri dishes. The same procedure was repeated for bulk drug.

Standard Preparation (Stress)

The first day standard of the Pyrazinamide was weighed and reground with dry KBr Using agate mortar and pestle. The KBr were prepared by using KBr pellets press instrument .Then the percentage transmittance of the

standard was measured .The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Bulk Preparation (stress)

The first day standard of the Pyrazinamide was weighed and reground with dry KBr Using agate mortar and pestle. The KBr were prepared by using KBr pellets press instrument .Then the percentage transmittance of the standard was measured .The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

The next day (1st) day, required sample has been taken from the Petri dish. The required amount of bulk drug was reground with dry KBr using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured. The spectrum obtained from degraded sample was compared with standard spectrum. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar

THERMAL DEGRADATION

Bulk Preparation (stress)

The next day (1st) day, the bulk from chamber was removed and the required quantity has been taken from the Petri dish. It was kept in the same place. The required amount bulk drug was reground with KBr agate mortar and pestle .The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured .The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample Preparation (stress)

Similar bulk procedure was followed

SUNLIGHT

Bulk Preparation (stress)

The next day (1st) day, the required quantity has been taken from the Petri dish .It was kept in the same place. The required amount bulk drug was reground with KBr agate mortar and pestle .The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured .The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample Preparation (stress)

Similar bulk procedure was followed

UHPLC –ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Ultra High Performance Liquid Chromatography

Ultra High Performance Liquid Chromatography is based on a modification to traditional liquid chromatographic systems that makes possible the use of increased operating pressures. Higher maximum operating pressures mean that packing material consisting of small (2µm) particle size can be used, by decreasing the particle size of packing material, the analyst can maximize the number of theoretical plates, making shorter column length possible. At the same time, by decreasing the particle size, the optimum range of mobile phase linear velocities is greatly expanded. As a result, higher linear velocities can be used without sacrificing separation quality. Both of these factors result in faster analytical run times and increased sample throughout.

UHPLC instrument consists of reservoir of mobile phase, a pump, an injector, a separation column, and detector, compounds are separated by injecting a plug of the sample mixture into the column. The different compounds in the mixture pass through the column at different rates due to difference in their partitioning behavior between the mobile liquid and the stationary phase.

Advantages of UHPLC

Decreases run time and increases sensitivity.

Provides the selectivity, and dynamic range of LC analysis

Maintaining resolution performance

Expands scope of multi residue methods

Less solvent consumption.[40]

Intraday scheme UHPLC-Study of Bulk and Formulation (Table: 5)

Degradation	Experimental conditions	Storage condition	Sampling time
	Control sample (No acid or base)	Room Temperature	90 mins
Hydrolysis	0.1M HCl	Room Temperature	90 mins
	0.1 M NaOH	Room Temperature	90 mins
	Acid control (no API)	Room Temperature	90 mins
	Base control (no API)	Room Temperature	90 mins
Oxidative	5% H_2O_2	Room Temperature	90 mins
	5% H_2O_2 (no API)	Room Temperature	90 mins

Interday scheme UHPLC-Study of Bulk and Formulation (Table: 6)

Degradation	Experimental conditions	Storage condition	Sampling time
	Control sample (No acid or base)	Room Temperature	3rd day
Hydrolysis	0.1 M HCl	Room Temperature	3rd day
	0.1M NaOH	Room Temperature	3rd day
	Acid control (no API)	Room Temperature	3rd day
	Base control (no API)	Room Temperature	3rd day
Oxidative	5%H_2O_2	Room Temperature	3rd day
	5%H_2O_2(no API)	Room Temperature	3rd day
Thermal	Heating chamber	50ºC	3rd day
Photolysis	Powder form	sunlight	3rd day

REAGENTS AND CHEMICALS USED FOR UHPLC

Acetonitrile

Distilled Water

Preparation of Phosphate Buffer (pH3.0)

Dissolved 6.8045gm of Potassium di hydrogen phosphate and add 1.844gm of Sodium hydroxide in 900 ml of water and pH adjusted to 3.0 with phosphoric acid and diluted to 1000ml with water. Then the solution was filtered through 0.45µ membrane filter.

Preparation of Mobile Phase:

A mixture of 100 volume of acetonitrile and 900 volume of phosphate buffer (100:900) respectively. The mobile phase was prepared and degassed. Mobile was used as diluent.

Standard Preparation:

80mg Pyrazinamide was transferred to 200ml volumetric flask and dissolved in distilled water and the volume made up to 200ml achieve a concentration of 4mg/ml. An aliquot solution was diluted with distilled water to get final concentration of 40µg/ml.

METHODOLOGY FOR PYRAZINAMIDE IN BULK AND FORMULATION BY UHPLC

System suitability solution: Transfer of 1ml hydrochloric acid to volumetric flask, dilute with standard preparation to volume mix. Keep the solution on a boiling water bath for 5 minutes and cooled. (1:4)

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed, transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 4mg/ml .An aliquot solution was diluted with water to get final concentration of 40 µg/ml.

Sample Preparation (stress)

80 mg equivalent of Pyrazinamide tablets were weighed, transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 4mg/ml .An aliquot solution was diluted with water to get final concentration of 40 µg /ml. Before the sample, bulk and standard solutions were filtered through a 0.45µm membrane filter.

Chromatographic Conditions:

Column	:	C18, 25x4.6mm, (1.7µ.)
Stationary phase	:	Silica
Elution type	:	Isocratic
Mobile phase	:	900:100(Phosphate buffer: acetonitrile)
Detector	:	PDA detector
Flow rate	:	1 ml/ minute
λ max	:	270nm
Injection volume	:	20µl

Determination of Retention Time

The mobile phase was injected first to determine the absence of any interference with the base line. The retention time was then determined by injecting 20µl of the standard in the column and the retention time was determined using 270 nm as the detection wavelength. The retention time was found to be 7 .0 to7.5 mins for pyrazinamide.

Analysis of sample solution:

The sample solution was diluted to get the required concentration and used for the quantification of Pyrazinamide 20 µl of the solution was injected into the column, retention time and peak area was determined.

Assay:

20µl of Standard and sample solutions were injected separately with the flow rate of 1mL/minutes of the mobile phase containing acetonitrile and phosphate buffer of 100:900 Proportions.

The amount of Pyrazinamide was calculated from the obtained chromatogram.

$$\text{Amount present} = \frac{\text{Spl. Area} \times \text{Std Wt.} \times \text{Dil .factor} \times \text{Avg .Wt.} \times \text{Purity of Std}}{\text{Std. Area} \times \text{Spl. Wt.} \times 100}$$

Percentage Content of Pyrazinamide

$$\text{Percentage Content} = \frac{\text{Amount to be present} \times 100}{\text{Label claim}}$$

INTRA DAY STUDY BY UHPLC OF BULK AND FORMULATION

Hydrolytic Degradation Using 0.1M NaOH

Standard Preparation

80mg of Pyrazinamide was weighed and transferred to 200ml volumetric flask. It was dissolved in distilled water and the volume made up to 200ml to achieve a concentration of 4mg/ml. An aliquot solution was diluted with distilled water to get final concentration of 40µg/ml.

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to a 200ml volumetric flask. It was dissolved in 0.1M NaOH and the volume made up to 200ml to achieve a concentration of 4mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml .The retention time and peak areas were determined by recording the chromatogram.

Sample Preparation (stress)

80mg equivalent of Pyrazinamide tablets were weighed and transferred to a 200ml volumetric flask. It was dissolved in 0.1M NaOH and the volume made up to 200ml to achieve a concentration of 4mg/ml. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ ml. The retention time and peak areas were determined by recording the chromatogram.

Blank Preparation

20ml of 0.1M NaOH was taken in a 200ml volumetric flask. After 90mins, an aliquot solution was diluted with mobile phase, blank were determined by the chromatogram. Before solution was injected in to the column

HYDROLYTIC DEGRADATION USING 0.1M HCl

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to 200ml volumetric flask. It was dissolved in 0.1M HCl and volume made up to 200 ml to achieve a concentration of 4mg/ml. after 90mins, an aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatograms.

Sample Preparation (stress)

80mg of Pyrazinamide tablets were weighed and transferred to 200ml volumetric flask. It was dissolved in 0.1M HCl and volume made up to 200ml using to achieve a concentration of 4mg/ml. after 90mins, an aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatograms

Blank Preparation

20ml of 0.1M HCl was taken in a 200ml volumetric flask. After 90mins, an aliquot solution was diluted with mobile phase, blank were

determined by the chromatogram. Before solution was injected in to the column.

OXIDATIVE DEGRADATION USING 5% H_2O_2

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to a 200ml of volumetric flask. It was dissolve in 5% H_2O_2 and the volume made up to 200ml using to achieve a concentration of 4mg/mL.After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatograms.

Sample Preparation (stress)

80mg equivalent of Pyrazinamide tablets were weighed and transferred to a 200ml volumetric flask. It was dissolve in 5% H_2O_2 and the volume made up to 200ml to achieve a concentration of 4mg/ml. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml .The retention time and peak area were determined by recording the chromatograms

Blank Preparation

20ml of 5% H_2O_2 was taken in a 100ml volumetric flask. After 90mins, an aliquot solution was diluted with mobile phase, blank were determined by the chromatogram. Before solution was injected in to the column

INTER DAY STUDY BY UHPLC OF BULK AND FORMULATION

HYDROLYTIC DEGRADATION USING 0.1M NaOH

Standard Preparation

80mg of Pyrazinamide was weighed and transferred to 200ml volumetric flask. It was dissolved in water and the volume made up to 200ml to achieve a concentration of 4mg/ml. An aliquot solution was diluted with distilled water to get final concentration 40µg/ml. Standard should be prepared daily.

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to a 200ml volumetric flask. It was dissolved in 0.1M NaOH and the volume made up 200ml to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatogram.

Sample Preparation (stress)

80mg equivalent of Pyrazinamide tablets were weighed and transferred to a 200ml volumetric flask. It was dissolved in 0.1M NaOH and the volume made up 200 ml to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatogram.

Blank Preparation

20ml of 0.1M NaOH was taken in a 200ml volumetric flask. After 90mins, an aliquot solution was diluted with mobile phase, blank were determined by the chromatogram. Before solution was injected in to the column.

HYDROLYTIC DEGRADATION USING 0.1M HCI

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to 200ml volumetric flask. It was dissolved in 0.1M HCl and volume made up to 200ml to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get final concentration of 40 µg/ml. The retention time and peak area were determined by recording the chromatograms.

Sample Preparation (stress)

80mg of Pyrazinamide tablets were weighed and transferred to 100ml volumetric flask. It was dissolved in 0.1M HCl and volume made up to 200ml

using to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatograms

Blank Preparation

20ml of 0.1M HCl was taken in a 100ml volumetric flask. After 90mins, an aliquot solution was diluted with mobile phase blank were determined by the chromatogram. Before solution was injected in to the column.

OXIDATIVE DEGRADATION USING 5% H_2O_2

Bulk Preparation (stress)

80mg of Pyrazinamide was weighed and transferred to a 200ml of volumetric flask. It was dissolved in 5% H_2O_2 and the volume made up to 200ml to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatogram.

Sample Preparation (stress)

80mg equivalent of Pyrazinamide tablets were weighed and transferred to a 200ml volumetric flask. It was dissolved in 5% H_2O_2 and the volume made up to achieve a concentration of 4mg/ml. Then the third day, an aliquot solution was diluted with mobile phase to get a concentration of 40µg/ml. The retention time and peak area were determined by recording the chromatograms

Blank Preparation

20ml of 5% H_2O_2 was taken in a 100ml volumetric flask. After 90mins an aliquot solution was diluted with mobile phase blank were determined by the chromatogram. Before solution was injected in to the column.

THERMAL DEGRADATION

Bulk Preparation (stress)

1gm of Pyrazinamide bulk was weighed and transferred to a Petri dish. This Petri dish was placed in a hot air oven at the temperature of 50ºC. The third day, 80mg pyrazinamide bulk was weighed from a Petri dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml.

Sample Preparation (stress)

1gm of Pyrazinamide tablets were weighed and transferred to a Petri dish. This Petri was placed in a hot air oven at the temperature of 50ºC. The third day, 80mg Pyrazinamide bulk was weighed from a Petri dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml

PHOTOLYTIC DEGRADATION USING SUNLIGHT

Bulk Preparation (stress)

1gm of Pyrazinamide bulk was weighed and transferred to a Petri dish. This Petri dish was placed under sunlight. The third day, 80mg Pyrazinamide bulk was weighed from a Petri dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration of 40 µg/ml.

Sample Preparation (stress)

1gm of Pyrazinamide bulk was weighed and transferred to a Petri dish. This Petri dish was placed under sunlight. The third day, 80mg Pyrazinamide bulk was weighed from a Petri dish and transferred to 200ml volumetric flask. An aliquot solution was diluted with mobile phase to get final concentration of 40µg/ml.

RESULTS AND DISCUSSION

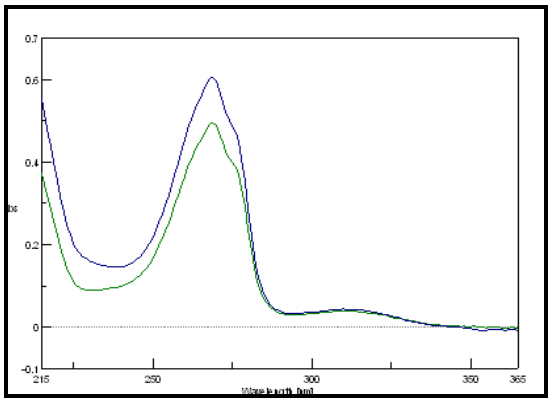
HYDROLYTIC DEGRADATION STUDY USING 0.1M SODIUM HYDROXIDE

Hydrolytic degradation study was carried out as per the procedure given in the material and methods .The assay value of active ingredients was calculated using UV spectrophotometry.

The respective UV- Spectrum and the values are given in Fig.1-4 and Table: 7, 8

Fig.1 Overlay spectrum of Pyrazinamide Bulk with Standard in 0.1M NaOH

30 Mins



90 Mins

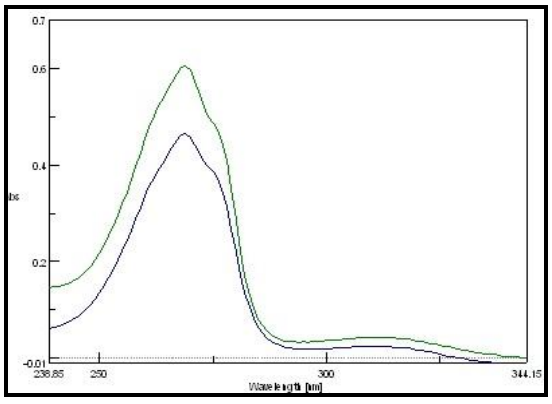
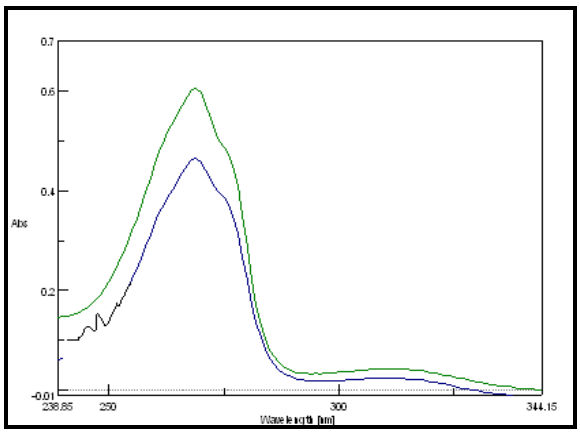


Fig.2 Overlay Spectrum of Pyrazinamide Sample with Standard in 0.1M NaOH

30 Mins



60 Mins

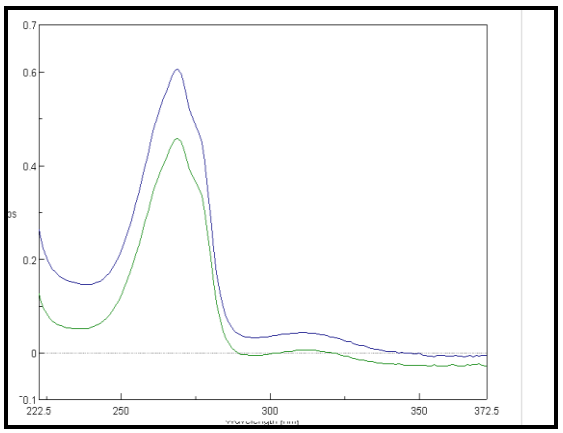
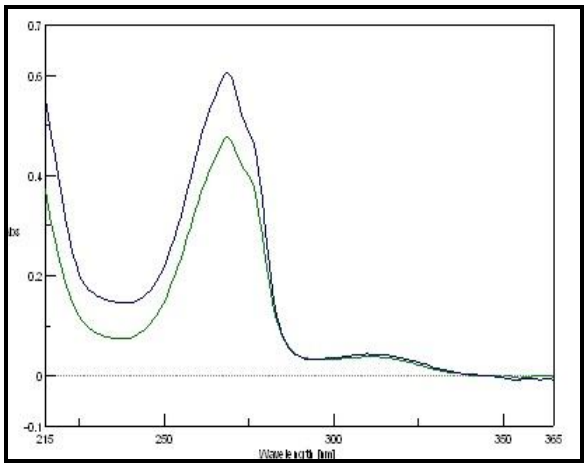


Fig: 3 Overlay Spectrum of Pyrazinamide Bulk with Standard in 0.1 M NaOH

1st Day



5th Day

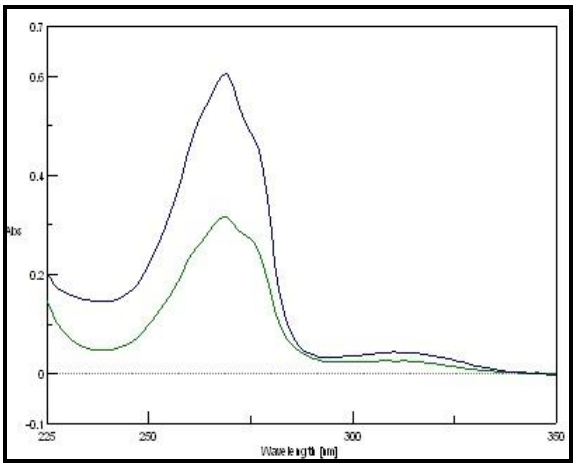
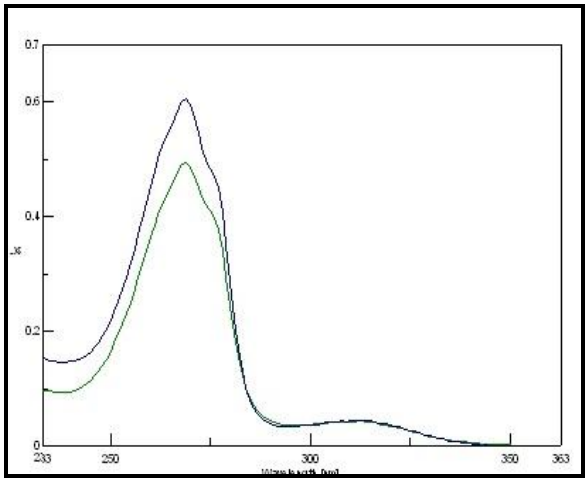


Fig: 4 Overlay Spectrum of Pyrazinamide Sample with Standard in 0.1 M NaOH

1st Day



5th Day

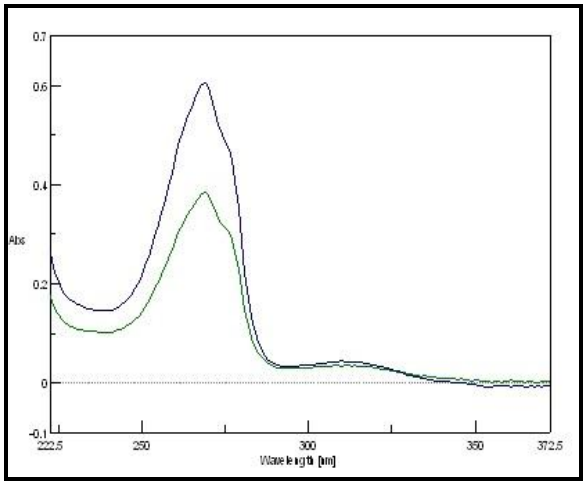


Table 7: Intra Day Results of Hydrolytic Degradation Using 0.1M NaOH

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.4870	0.6050	30 mins
		0.4868		
		0.4852		
2	Sample	0.4824	0.6050	30 mins
		0.4772		
		0.4614		
3	Bulk	0.4587	0.6050	60mins
		0.4572		
		0.4563		
4	Sample	0.4441	0.6050	60mins
		0.4434		
		0.4324		
5	Bulk	0.4587	0.6051	90mins
		0.4527		
		0.4535		
6	Sample	0.4431	0.6051	90mins
		0.4223		
		0.4200		

Table.7.1: Results Obtained From Hydrolytic Degradation 0.1M NaOH

Stress Condition (Alkali Hydrolysis)	**Time**	**Bulk Percentage Content (%)**	**Sample Percentage Content (%)**
0.1 M Sodium Hydroxide	30mins	80.38	78.29
	60mins	75.88	72.72
	90mins	75.20	70.82

Each value is the mean of three determinations.

Table.8 Inter Day Result of Hydrolytic Degradation Using 0.1M NaOH

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.4948	0.6050	1st day
		0.4925		
		0.4923		
2	Sample	0.4724	0.6051	1st day
		0.4722		
		0.4732		

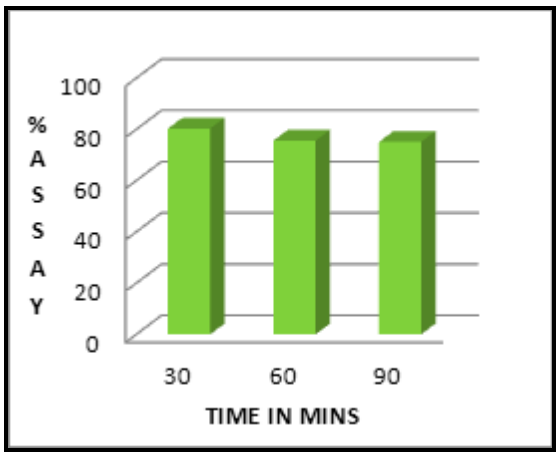
Table.8.1 Results Obtained From Hydrolytic Degradation 0.1 M NaOH

Stress Condition (Alkali Hydrolysis)	Time	Bulk Percentage Content (%)	Sample Percentage Content (%)
0.1M Sodium Hydroxide	1st Day	18.85	22.24
	3rd day	0	0
	5th day	0	0

GRAPHICAL REPRESENTATION OF INTRA DAY AND INTERDAY STUDY (0.1M NaOH)

Fig: 5 Assay values of Bulk and Samples at various time intervals

Bulk



Sample

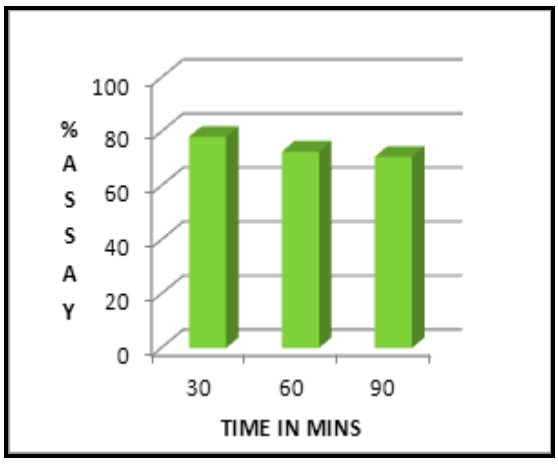
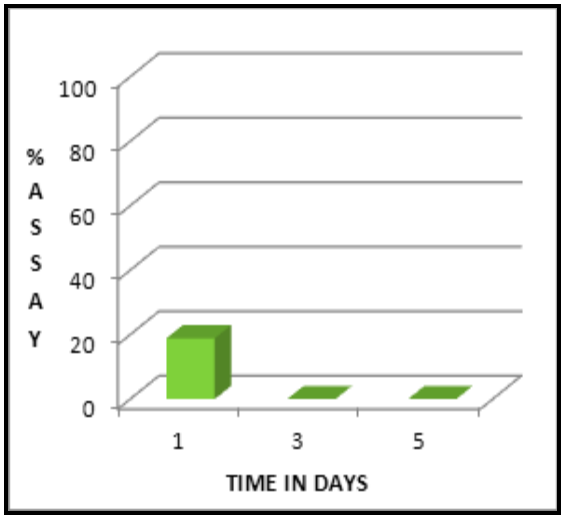
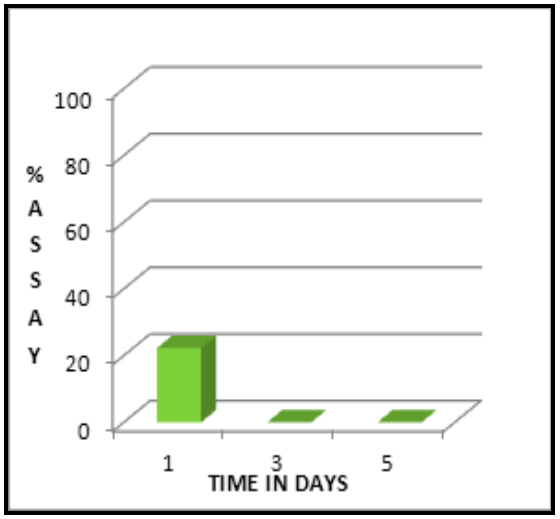


Fig: 6 Assay values of Bulk and Sample at Various Time Intervals

Bulk



Sample



The study results indicated that Pyrazinamide was unstable in alkali condition. Table 7 Shows the results of intraday degradation and the assay values of standard and sample.

In intraday investigation, the sample undergoes greater degradation. When compared to standard and it is clearly seen in assay values. The assay values of standard and sample were found to be 70.82% and 75.20% respectively at end of 90 mins degradation. The table 8.1 shows that the results of interday degradation. In interday study, both standard and sample have undergone maximum degradation on 1st day. The assay values of standard and sample were found to be 18.85% and 22.24% repectively. Complete degradation was observed from 3rd day onwards.

HYDROLYTIC DEGRADATION USING 0.1M HYDROCHLORIC ACID

Hydrolytic degradation study was carried out as per the procedure given the material and methods .The assay value of active ingredients was calculated using UV spectrophotometry. The respective UV- spectrum and the values are given in Fig.7-10, Table.9-10.

Fig: 7 Overlay spectrum of Pyrazinamide Bulk with Standard in 0.1M HCl

30 Mins 60 Mins

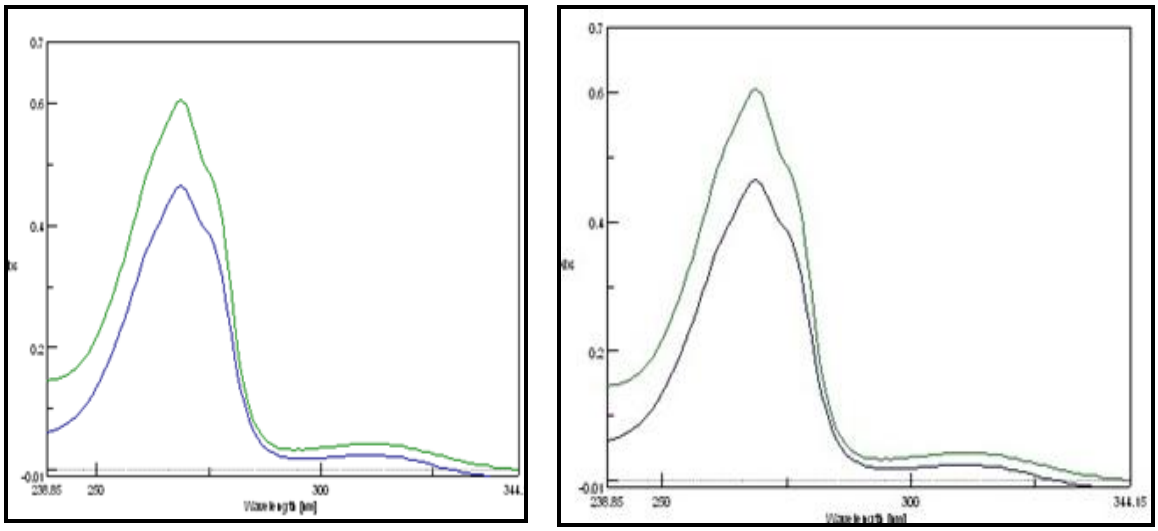


Fig: 8 Overlay spectrum of Pyrazinamide sample with Standard in 0.1M HCl

30 Mins 90 Mins

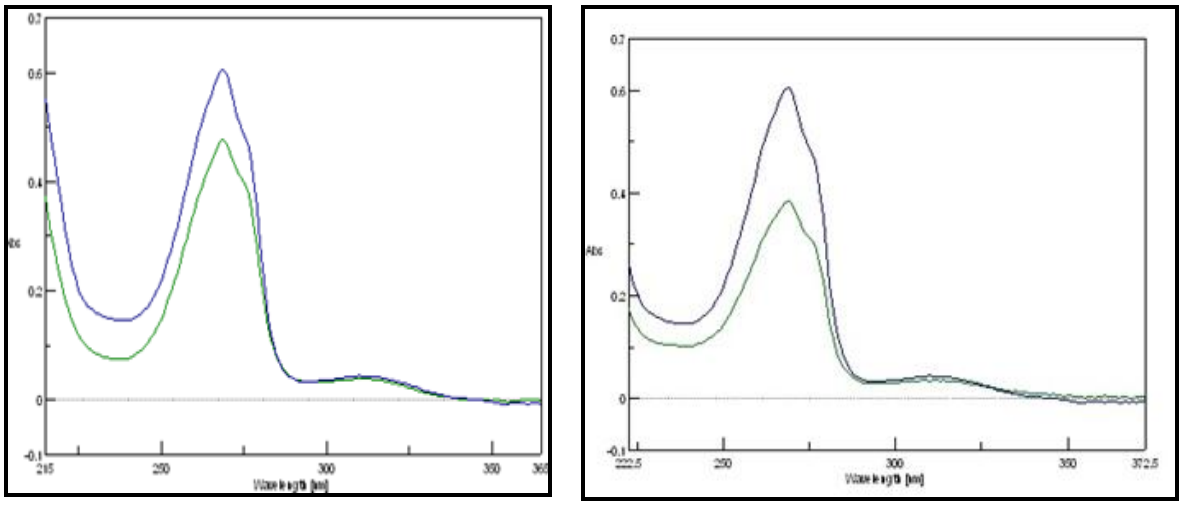
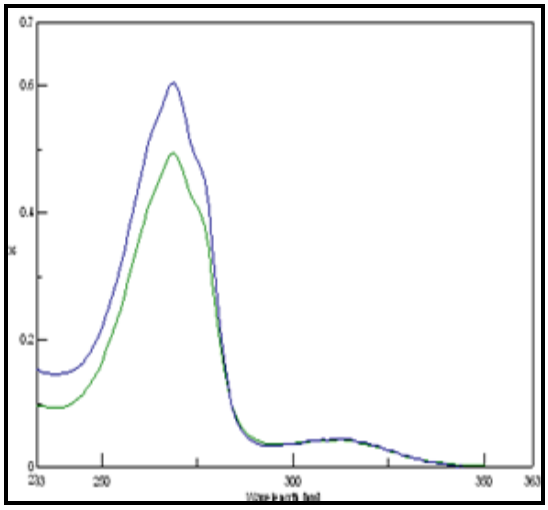


Fig: 9 Overlay spectrum of Pyrazinamide Bulk with Standard in 0.1M HCl

1st Day



5th Day

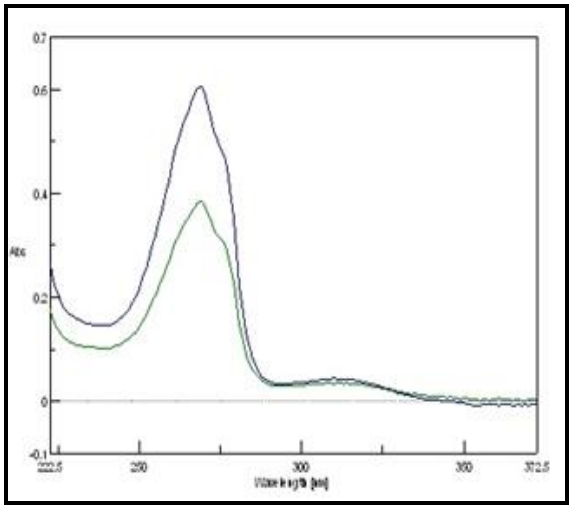
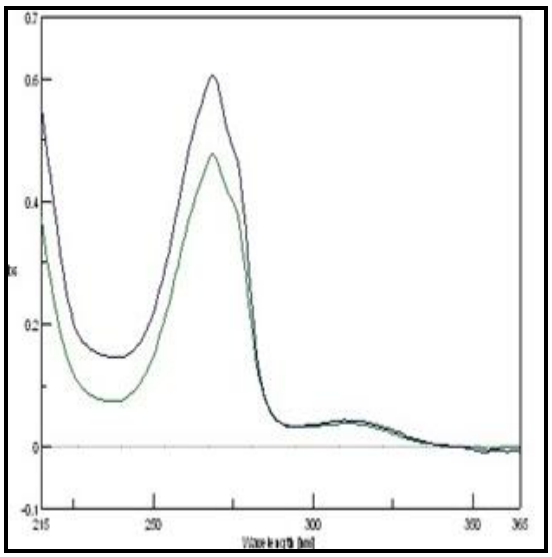


Fig.10 Overlay spectrum of Pyrazinamide Sample with Standard in 0.1M HCl

1st Day



5th Day

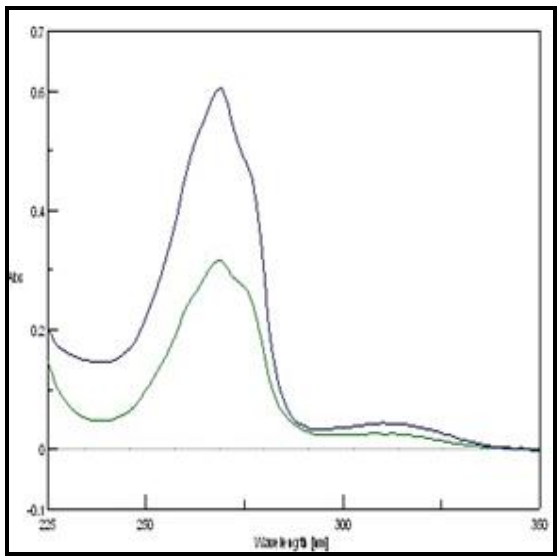


Table.9 INTRA DAY RESULTS OF HYDROLYTIC DEGRADATION USING 0.1M HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.4537	0.6051	30 mins
		0.4614		
		0.4632		
2	Sample	0.4258	0.6051	30 mins
		0.4261		
		0.4234		
3	Bulk	0.4513	0.6045	60mins
		0.4482		
		0.4491		
4	Sample	0.4275	0.6045	60mins
		0.4251		
		0.4202		
5	Bulk	0.4212	0.6045	90mins
		0.4110		
		0.4131		
6	Sample	0.4240	0.6045	90mins
		0.4193		
		0.4120		

Each value is the mean of three determinations

Table.9.1 Results Obtained From Hydrolytic Degradation 0.1M HCl

Stress Condition (Acid Hydrolysis)	Time	Bulk Percentage Content (%)	Sample Percentage Content (%)
0.1 M Hydrochloric Acid	30mins	75.90	70.20
	60mins	74.30	70.18
	90mins	68.61	69.16

Table.10 Inter Day Result Of Hydrolytic Degradation Using 0.1 M HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.4614	0.6005	1st day
		0.4582		
		0.4583		
2	Sample	0.4464	0.6005	1st day
		0.4462		
		0.4425		

Each value is the mean of three determinations

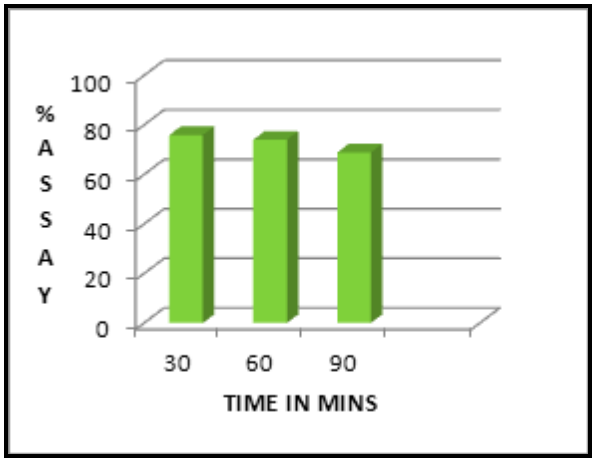
Table.10.1 Results Obtained From Hydrolytic Degradation 0.1M HCl

Stress Condition (Acid Hydrolysis)	Time	Bulk percentage content (%)	Sample percentage Content (%)
0.1 M Hydrochloric Acid	1st Day	23.13	26.77
	3rd Day	0	0
	5th Day	0	0

GRAPHCAL REPESENATION OF INTRA DAY AND INTERDAY STUDY (0.1M HCl)

Fig: 11 Assay values of Bulk and Sample various time intervals

Bulk



Sample

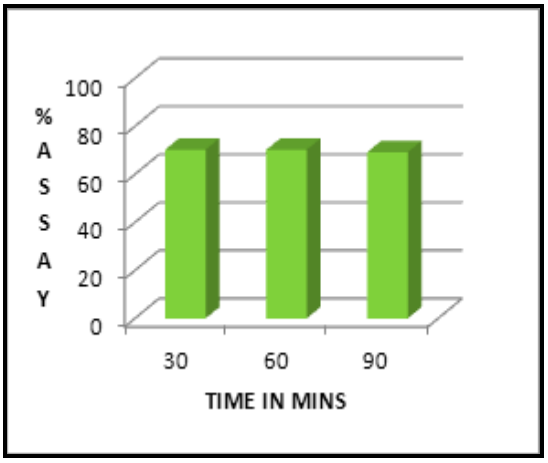
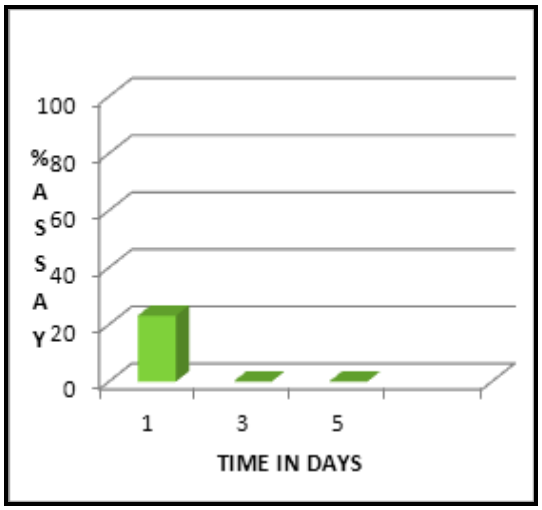
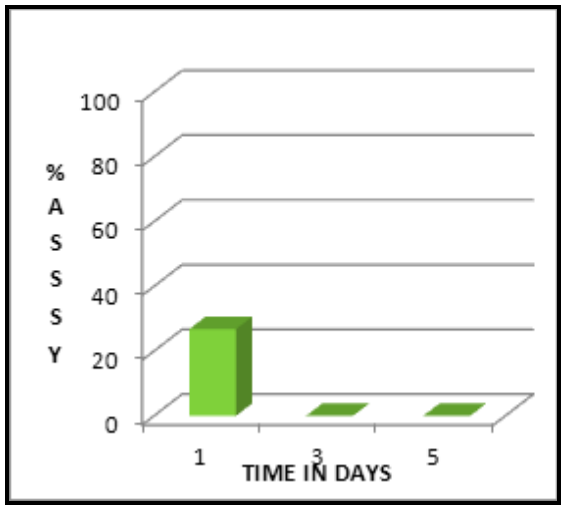


Fig: 12.Assay values of Bulk and Sample at various time intervals

Bulk



Sample



Hydrolytic acid degradation indicated that Pyrazinamide was unstable in acid condition .In intraday degradation both the standard and sample exhibits meagre amount of degradation. However sample undergoes greater degradation than standard at 90mins. The assay values of standard and sample was found to be 68.61% and 69.16% respectively. Interday study complete degradation was observed on both standard and sample from 3rd day on wards.

OXIDATIVE DEGRADATION USING 5% HYDROGEN PEROXIDE

Oxidative degradation study was carried out as per the procedure given in the material and methods .The assay value of active ingredients was calculated using UV Spectrophotometry.

The respective UV -spectrum and the values are given in Fig.13-16, Table-11-12

Fig .13 Overlay spectrum of Pyrazinamide Bulk with Standard in 5% H_2O_2

30 Mins 60 Mins

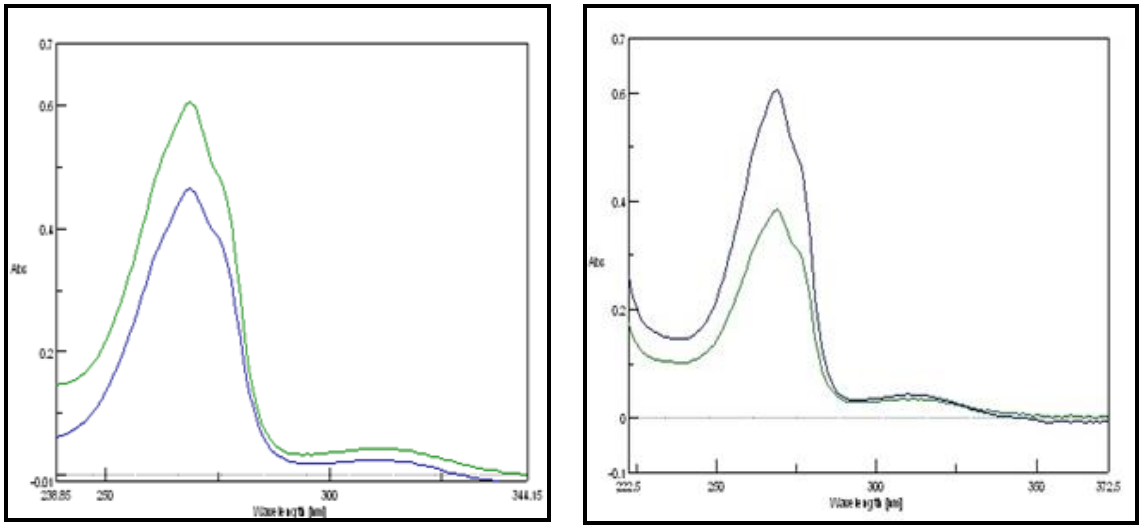


Fig.14 Overlay spectrum of Pyrazinamide Sample with Standard in 5% H_2O_2

30 Mins 90 Mins

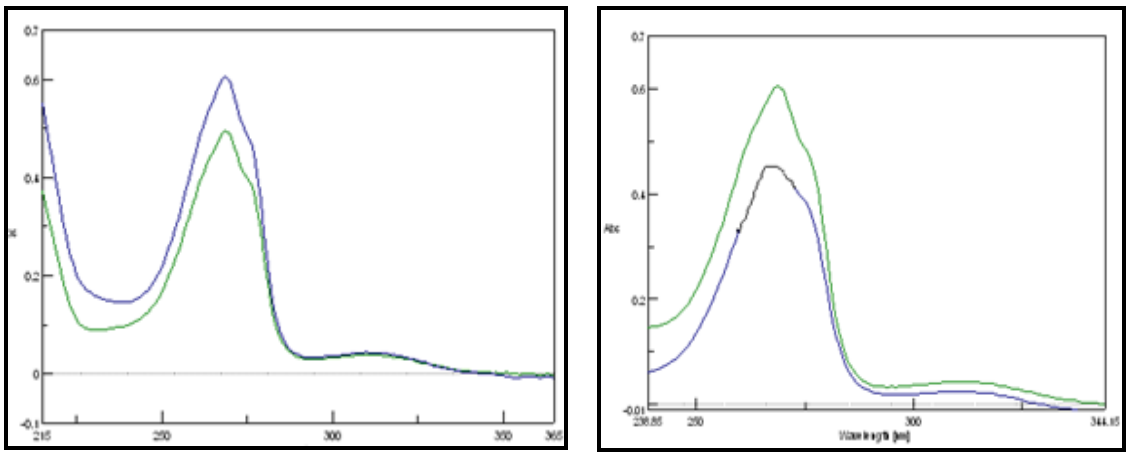
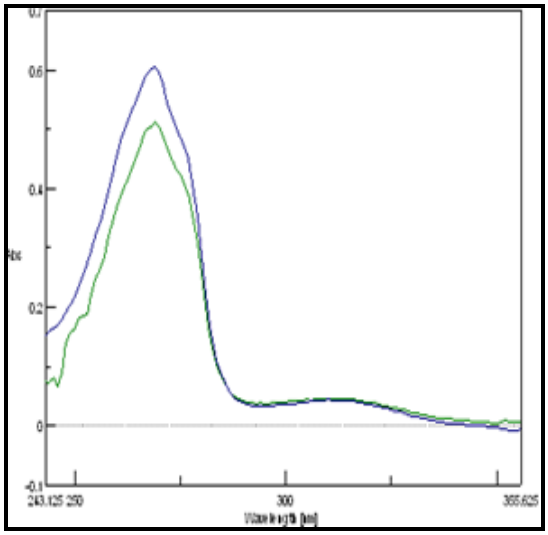


Fig.15.Overlay spectrum of Pyrazinamide Bulk with Standard in 5% H_2O_2

1st Day



5th Day

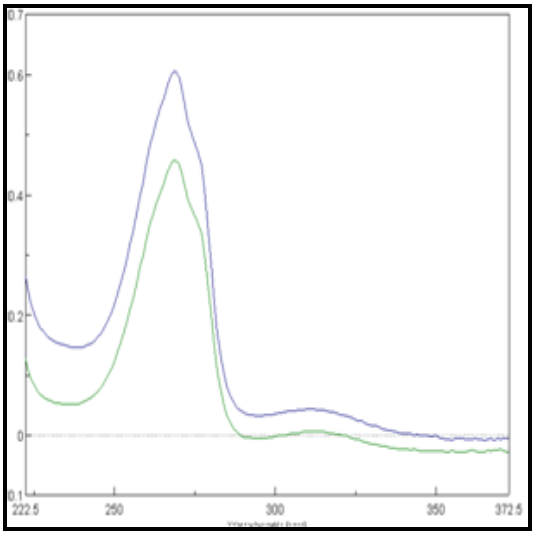
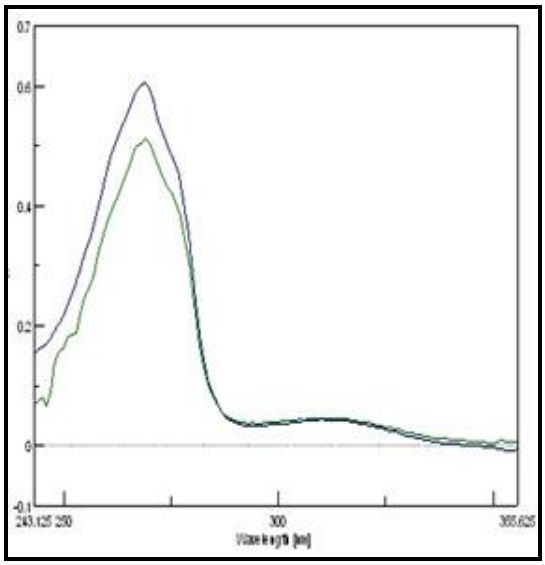


Fig.16.Overlay spectrum of Pyrazinamide Sample with Standard in 5%H_2O_2

1st Day



5th Day

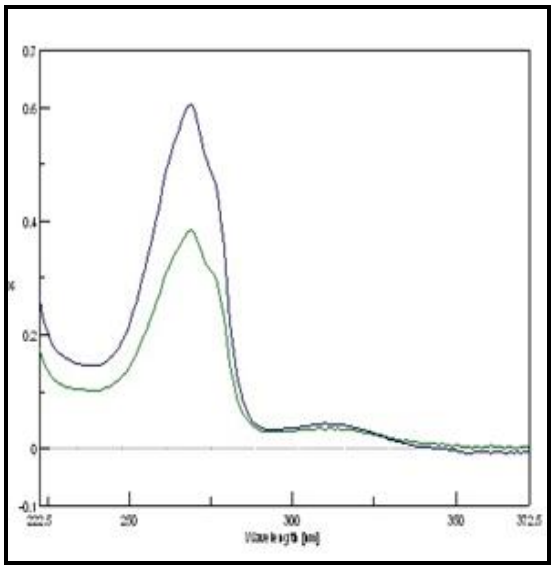


Table.11 Intra Day Results Of Oxidative Degradation Using 5% H_2O_2

S. No.	D rug	Absorbance	Standard	Time
1	Bulk	0.5282	0.6051	30 mins
		0.5321		
		0.5242		
2	Sample	0.4663	0.6051	30 mins
		0.4632		
		0.4544		
3	Bulk	0.4724	0.6050	60mins
		0.4712		
		0.4744		
4	Sample	0.4598	0.6050	60mins
		0.4588		
		0.4596		
5	Bulk	0.4591	0.6053	90mins
		0.4580		
		0.4520		
6	Sample	0.4563	0.6053	90mins
		0.4453		
		0.4363		

Table .11.1 Results Obtained from Oxidative Degradation 5% H_2O_2

Stress Condition (Oxidation)	Time	Bulk Percentage content (%)	Sample Percentage Content (%)
5% H_2O_2	30mins	87.30	76.24
	60mins	78.16	75.93
	90mins	75.4	73.71

Table .12 Inter Day Result Of Oxidative Degradation Using 5% H_2O_2

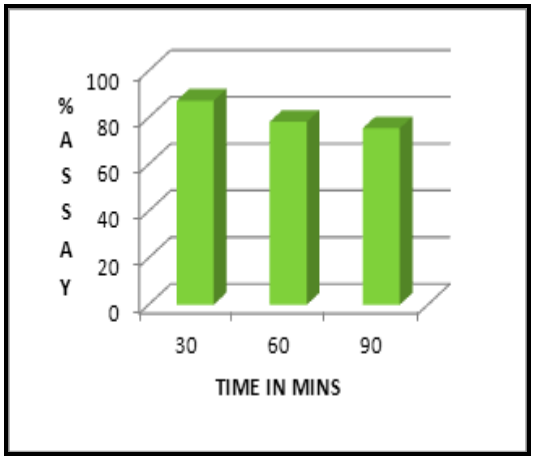
S.No.	D rug	Absorbance	Standard	Time
1	Bulk	0.4831	0.5983	1st day
		0.4821		
		0.4820		
2	Sample	0.4851	0.5983	1st day
		0.4821		
		0.4800		

Table .12.1Results Obtained From Oxidative Degradation 5% H_2O_2

Stress Condition (Oxidation)	Time	Bulk Percentage Content (%)	Sample Percentage Content (%)
5% H_2O_2	1^{st} Day	20.63	20.63
	3^{rd} Day	0	0
	5^{th} Day	0	0

Fig: 17 .Assay Values of Bulk and Samples at Various Time Intervals (5%H_2O_2)

Bulk



Sample

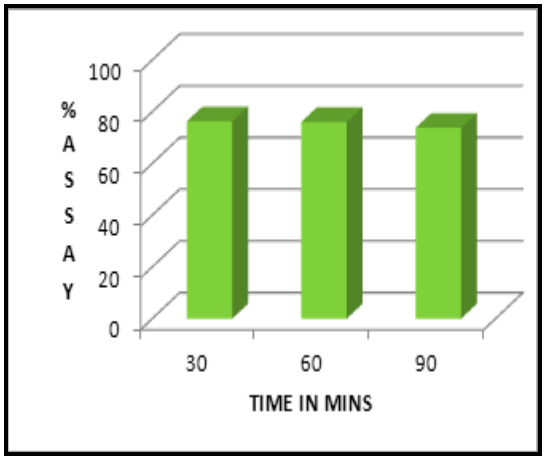
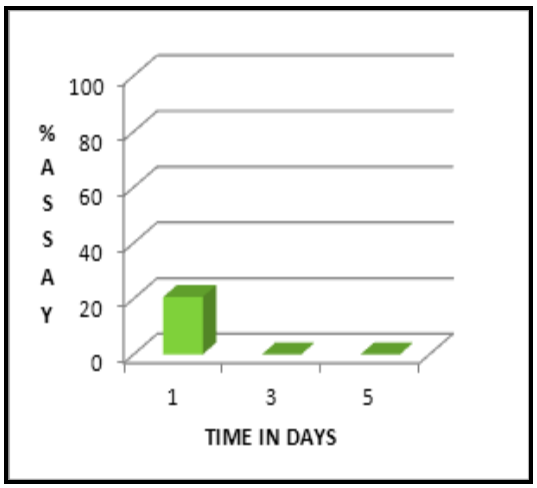
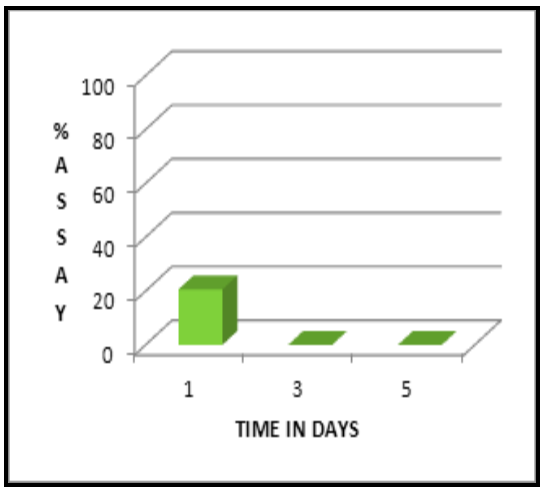


Fig.18. Assay values of Bulk and samples at various time intervals

Bulk



Sample



Oxidative degradation shows that Pyrazinamide was found to have a mild degradation in oxidative condition. In intraday analyses both sample and standard have undergone extensive degradation but when compared to standard, sample was largely degraded, at the end of 90mins degradation. In inter-day degradation study on 1st day standard and sample undergone maximum degradation. The assay values of standard and sample were 20.63% and 20.63% respectively. Complete degradation was seen from 3rd day onwards.

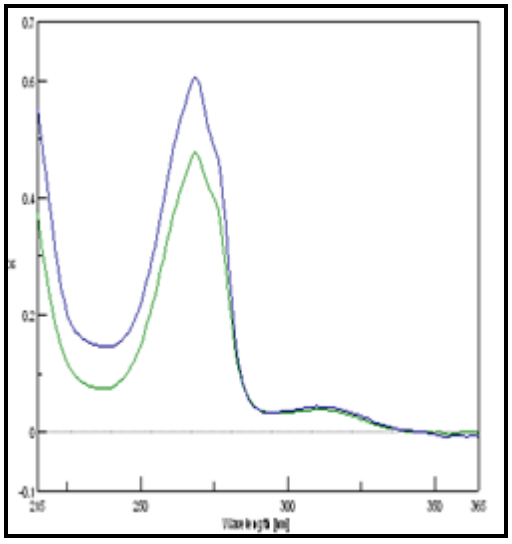
THERMAL DEGRADATION

Pyrazinamide standard and sample kept in heating chamber at 50˚C.The standard and samples powders were collected at different time intervals and the assay values were calculated by UV

Spectroscopy. The respective UV- spectrum and the values are given Fig.19-20, Table.13.

Fig .19. Overlay spectrum of Pyrazinamide Bulk with Standard

1st Day



5th Day

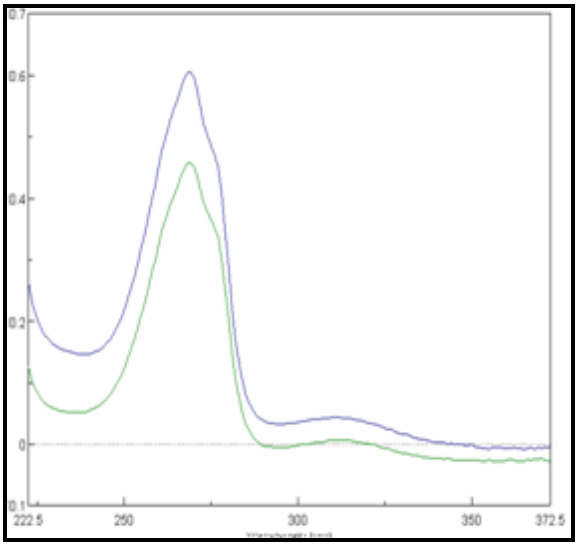
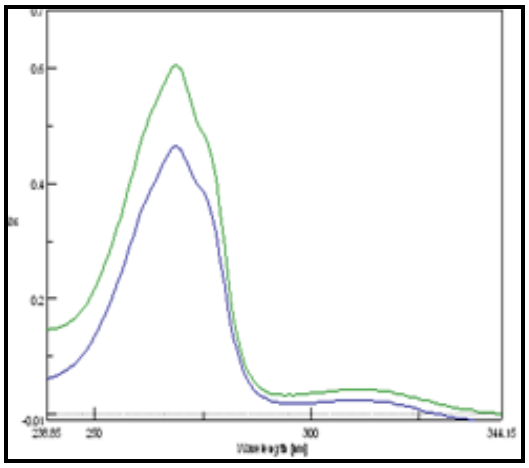


Fig.20. Overlay spectrum of Pyrazinamide Sample with Standard

1st Day



5th Day

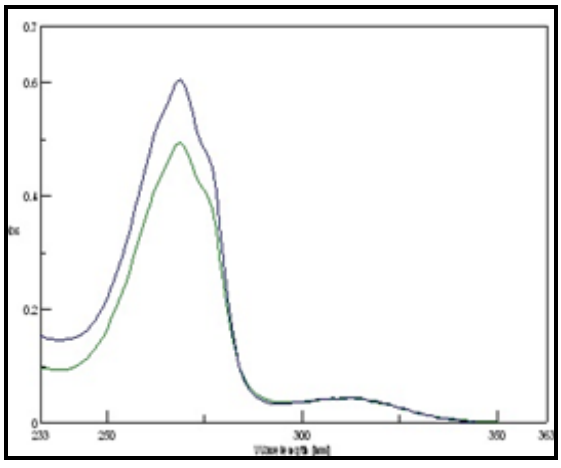


Table .13 Absorbance values for Thermal Degradation at 50˚C

S. No.	D rug	Absorbance	Standard	Time
1	Bulk	0.4626	0.6050	1st day
		0.4632		
		0.4652		
2	Sample	0.4542	0.6050	1st day
		0.4538		
		0.4533		
3	Bulk	0.4452	0.6052	3rd day
		0.4432		
		0.4430		
4	Sample	0.4442	0.6052	3rd day
		0.4400		
		0.4418		
5	Bulk	0.4428	0.6052	5th day
		0.4432		
		0.4300		
6	Sample	0.4420	0.6052	5th day
		0.4400		
		0.4312		

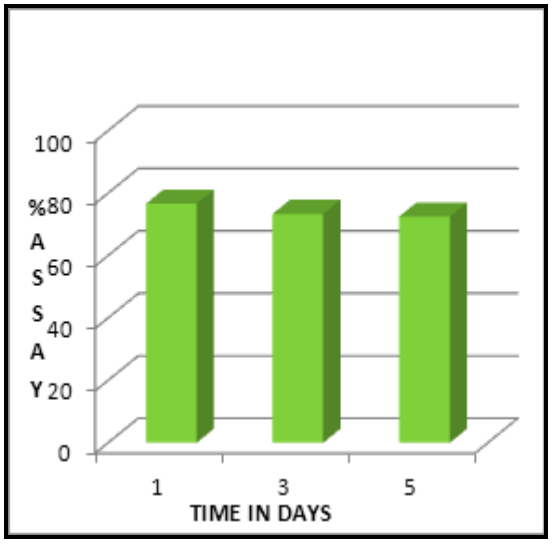
Table .13.1Results Obtained from Thermal Degradation at 50˚C

Stress Condition (Thermal)	Time	Bulk percentage content (%)	Sample percentage Content (%)
50˚C	1st day	76.63	75.00
	3rd day	73.35	73.03
	5th day	72.48	72.32

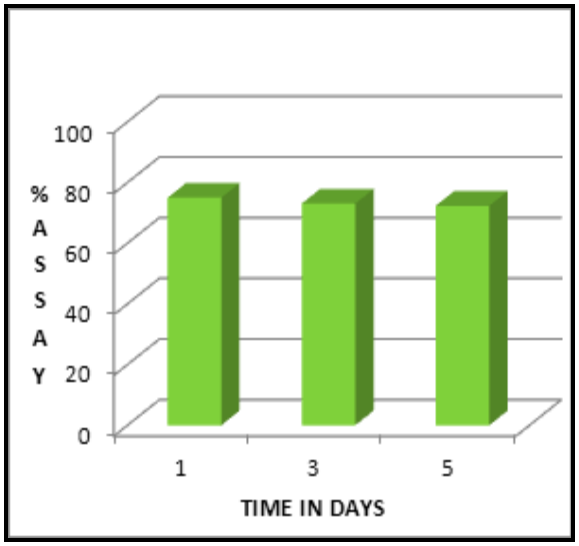
GRAPHICAL REPRESENSATION OF THERMAL DEGRADATION STUDY

Fig .21. Assay values of Bulk and Sample at various time intervals

Bulk



Sample



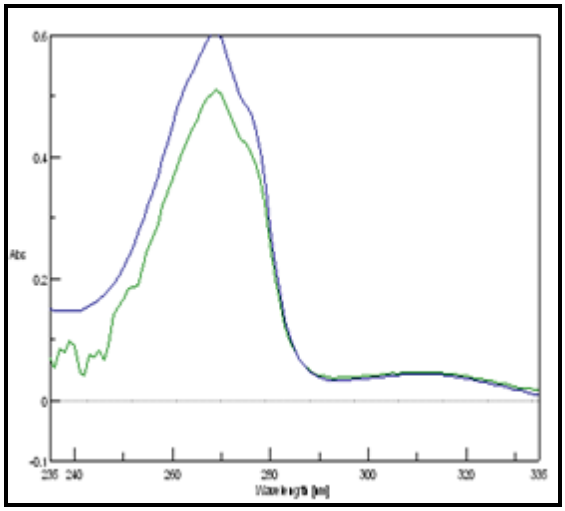
In thermal degradation study small amount of degradation was observed up to 5th day .It shows that bulk and formulation exhibit stability against temperature effect. At the end of the 5th day the assay value bulk and sample were found to be 72.50% and 72.35% respectively.

PHOTOLYTIC DEGRADATION USING SUNLIGHT

Pyrazinamide standard and sample were kept in heating chamber at 50˚C.The standard and sample powders were collected at different time intervals and the assay value were calculated by UV Spectroscopy. The respective UV spectrum and the values are given Fig .22-23 Table-14.

Fig.22. Overlay spectrum of pyrazinamide Bulk with Standard

1st Day



5th Day

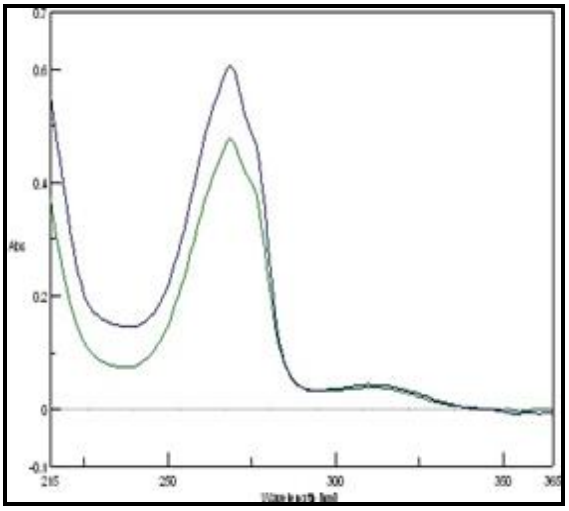
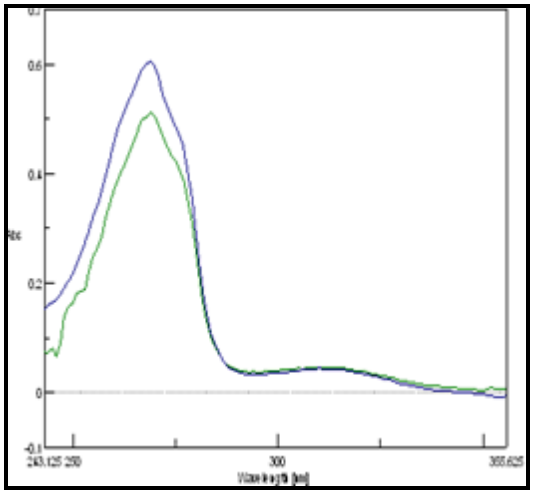


Fig.23. Overlay spectrum of pyrazinamide sample with Standard

1st Day



5th Day

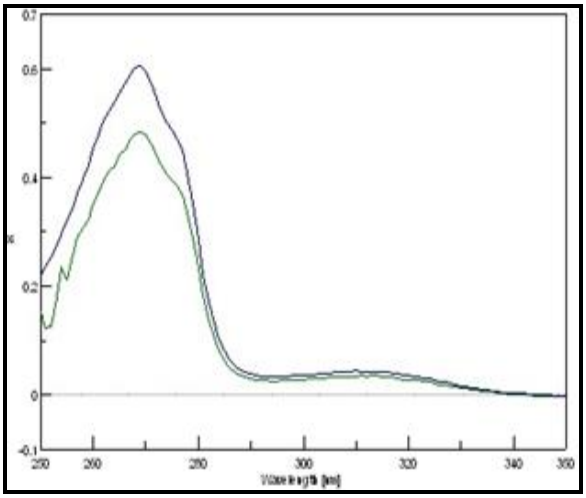


Table. 14. Absorbance Values for Photolytic Degradation

S. No.	D rug	Absorbance	Standard	Time
1	Bulk	0.4681	0.6050	1st day
		0.4652		
		0.4600		
2	Sample	0.4623		
		0.4612		
		0.4620		
3	Bulk	0.4589	0.6050	3rd day
		0.4515		
		0.4500		
4	Sample	0.4523		
		0.4452		
		0.4442		
5	Bulk	0.4452	0.6050	5th day
		0.4432		
		0.4358		
6	Sample	0.4389		
		0.4385		
		0.4370		

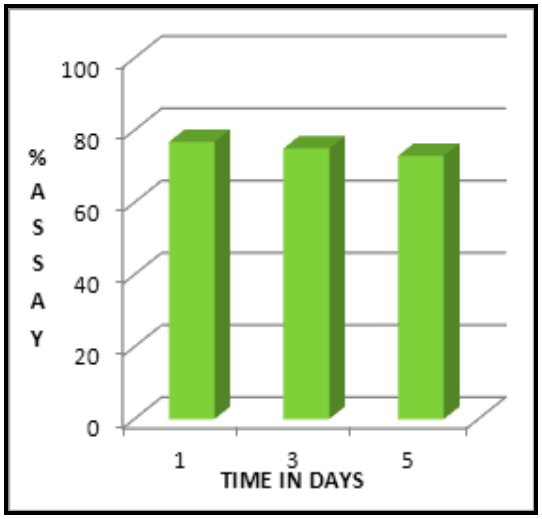
Table.14.1 Results Obtained from Photolytic Degradation

Stress Condition (Photolytic)	Time	Bulk percentage content (%)	Sample percentage Content (%)
Sunlight	1st day	76.76	76.33
	3rd day	74.95	73.92
	5th day	72.95	72.41

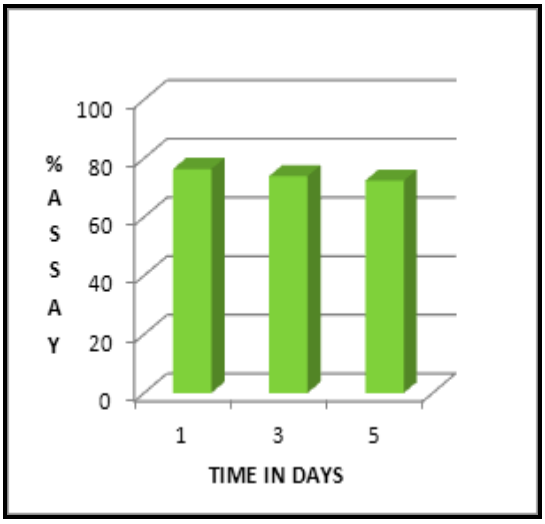
GRAPHICAL REPRESENTATION OF PHOTOLYTIC DEGRADATION STUDY

Fig .24. Assay values of bulk and samples at various time intervals

Bulk



Sample



Photolytic degradation studies shows that sample underwent greater degradation compared to bulk. The assay value of standard and sample were found to be 72.95% and 72.41% at the end of 5th day degradation.

2. IR STUDY OF BULK AND SAMPLES –UNDER STRESS AND NORMAL CONDITION:

In inter day Thermal and Photolytic degradation the samples were kept in heating chamber and sunlight. The next day the samples and ground with KBr. Then the KBr pellets were formed using pellet pressing technique.IR Spectrum was taken for pellets .IR spectrum is given in

Fig: 25-34, Table-15.

Fig.25. STANDARD

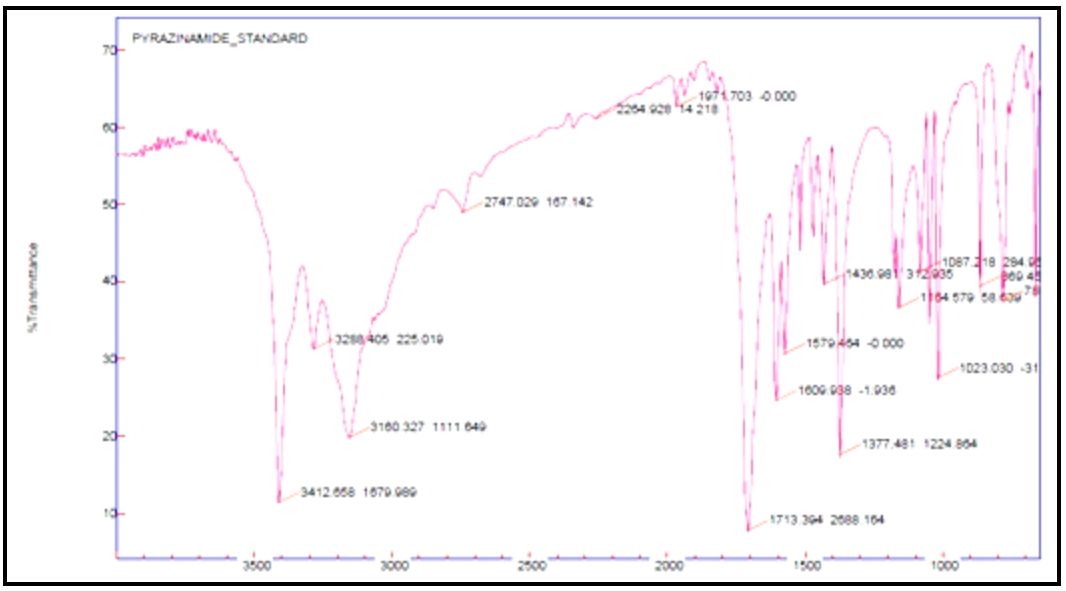
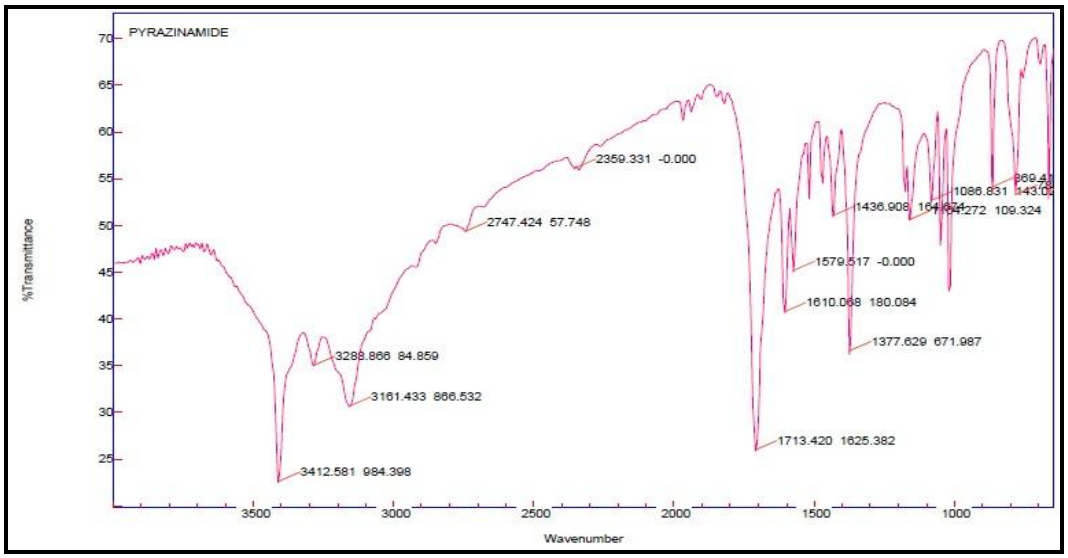


Fig.26. SAMPLE



THERMAL DEGRADATION

Fig.27.Overlay IR Spectrum of Standard with Bulk in 1st Day

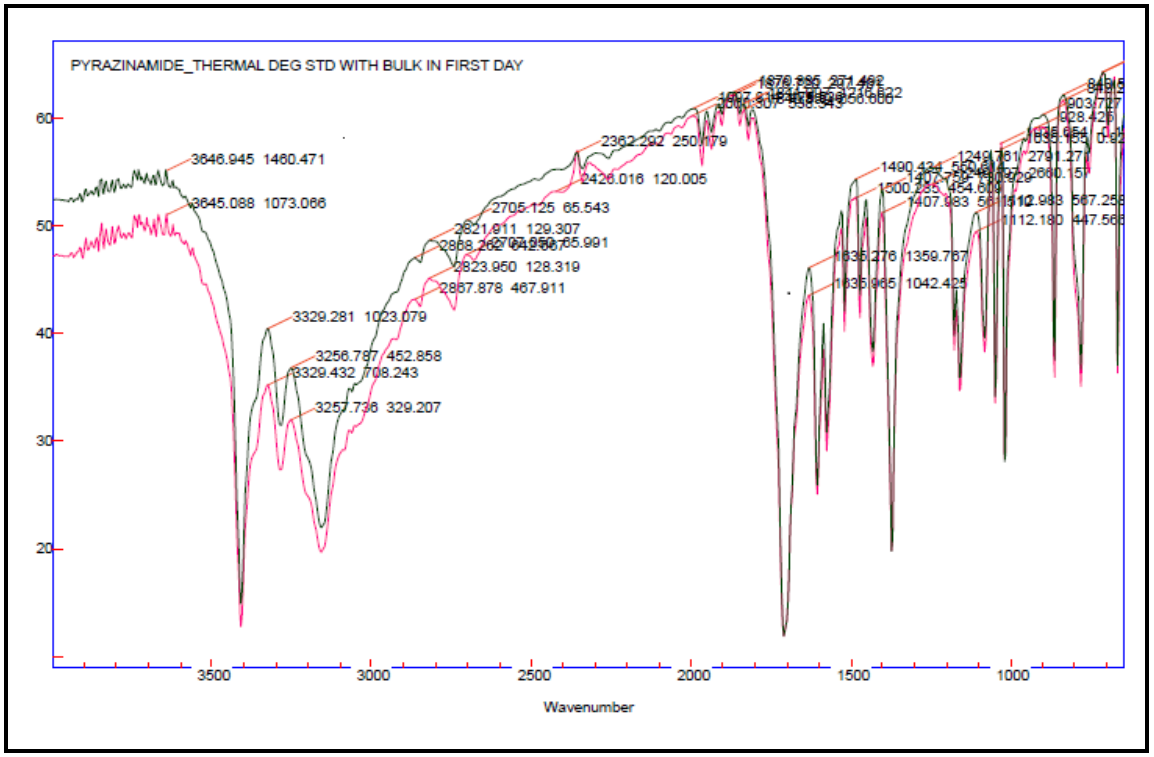


Fig .28. Overlay IR Spectrum of Standard with Bulk in 5thDay

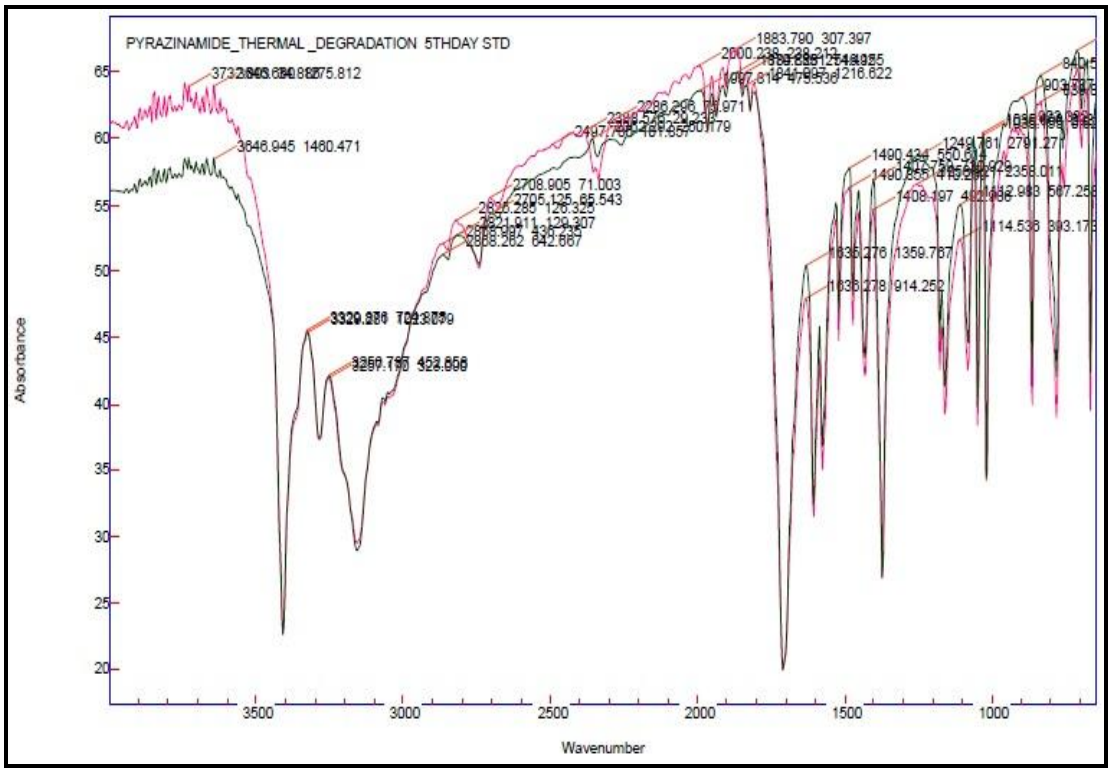


Fig .29. Overlay IR Spectrum of Standard with Sample in 1st Day

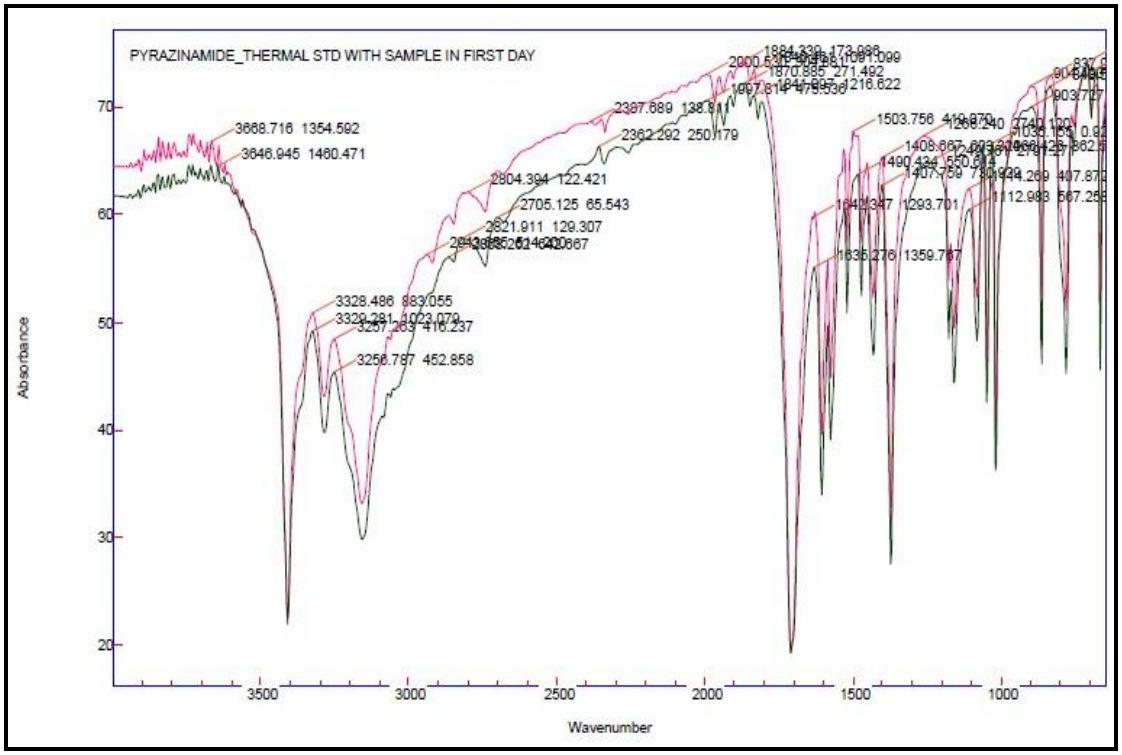
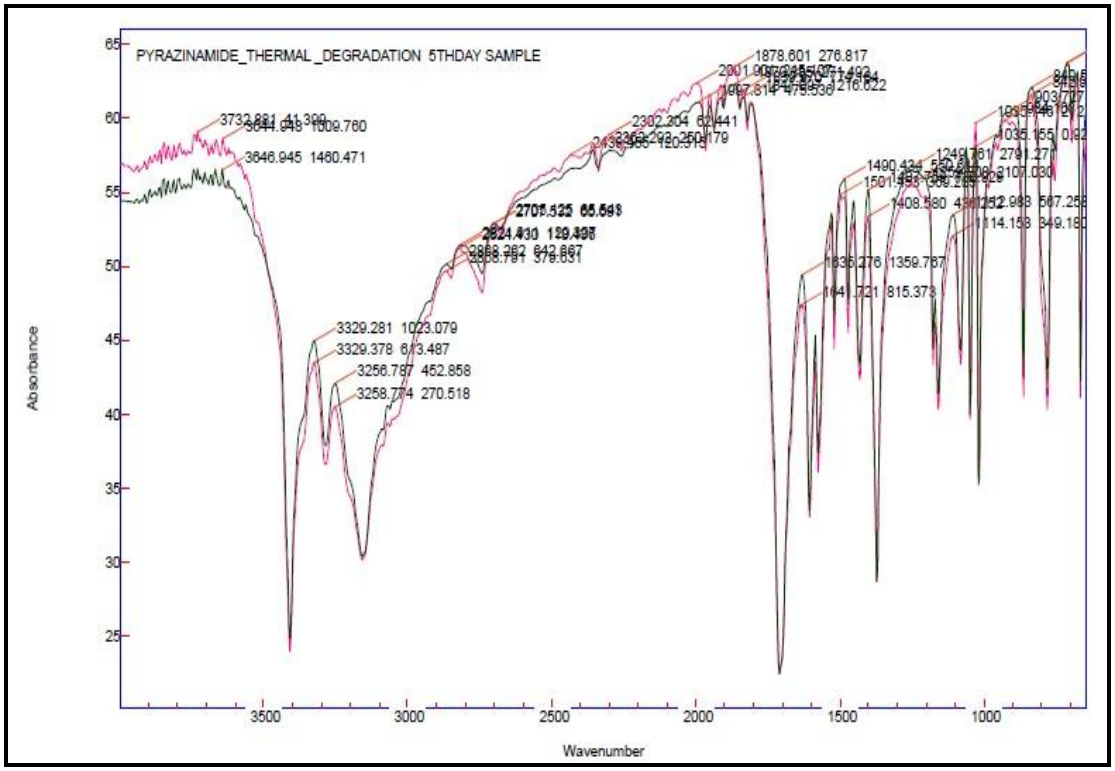


Fig.30. Overlay IR Spectrum of Standard with Sample in 5th Day



PHOTOLYTIC DEGRADATION USING SUNLIGHT

Fig.31. Overlay IR Spectrum of Standard with Bulk in 1st Day

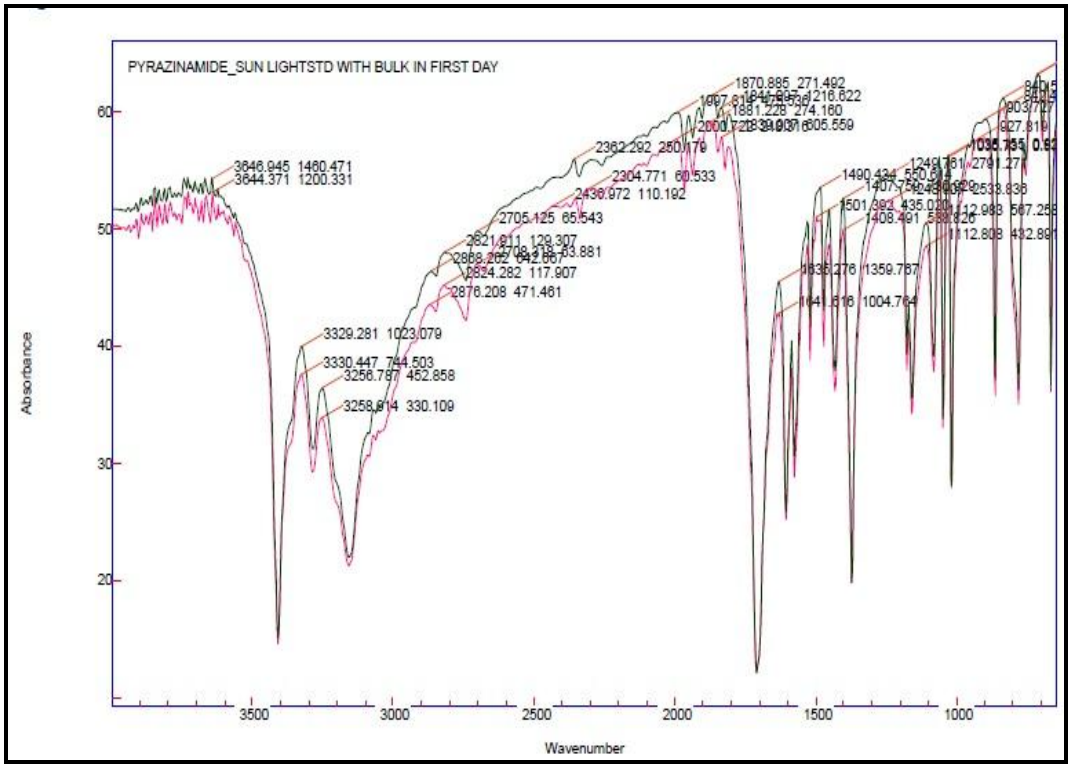


Fig.32. Overlay IR Spectrum of Standard with Bulk in 5thDay

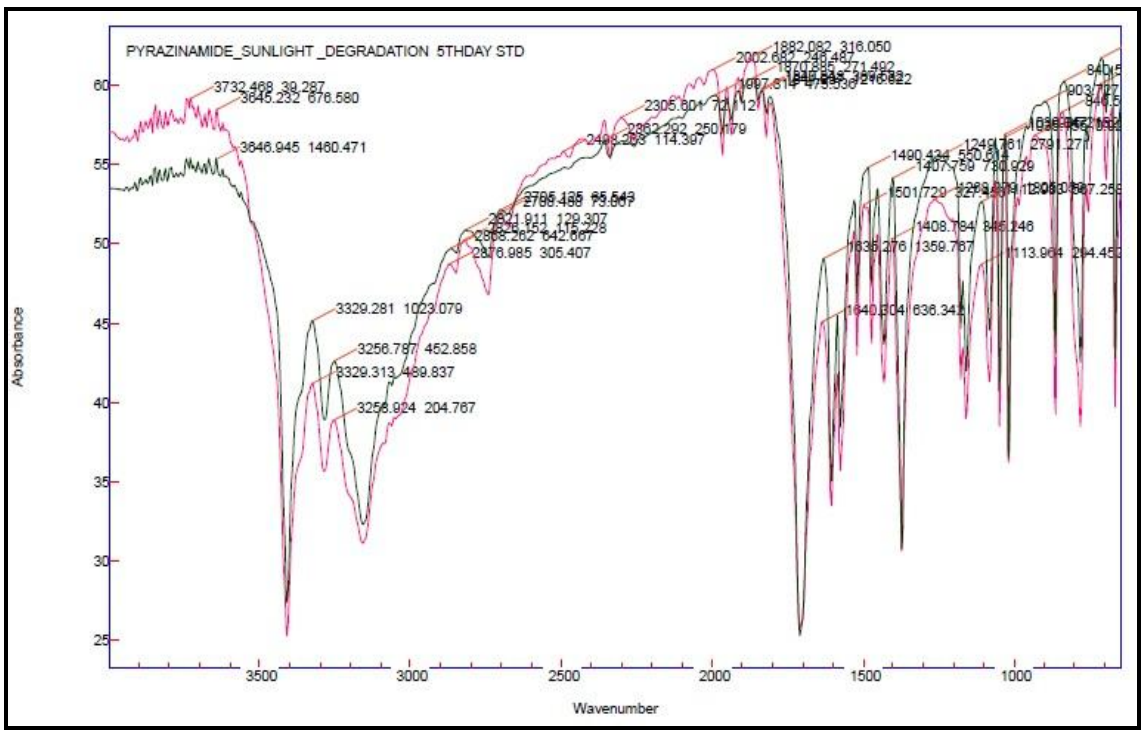


Fig.33.Overlay IR Spectrum of Standard with Sample in 1st Day

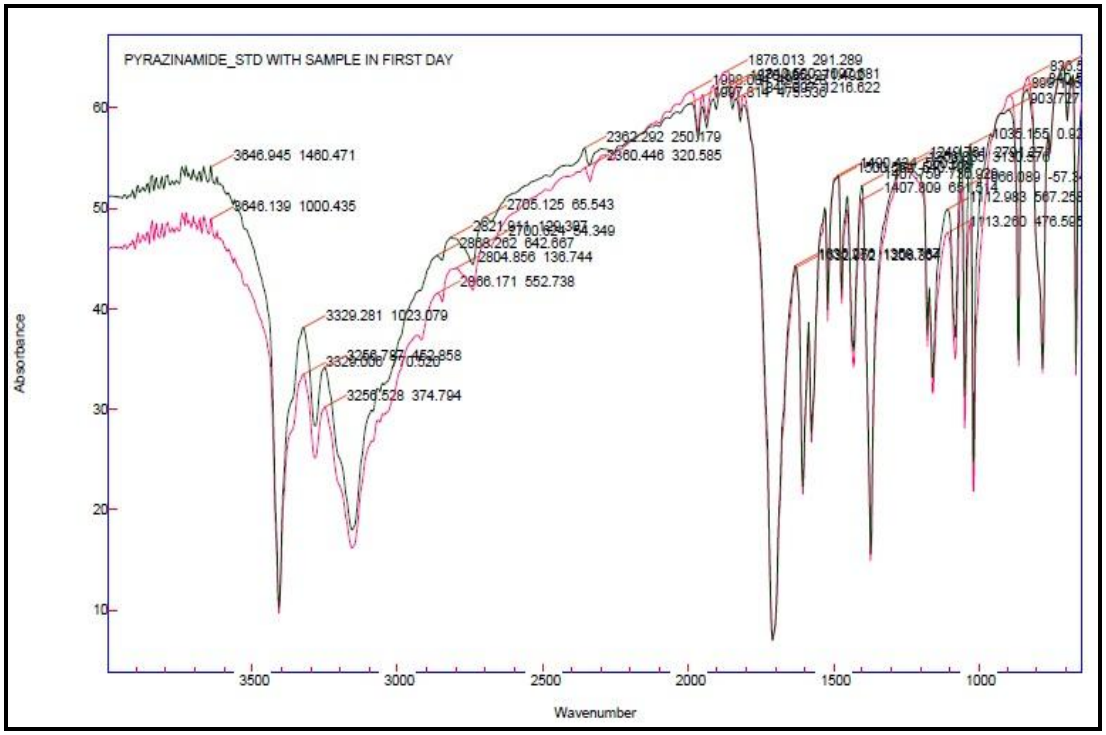


Fig.34. Overlay IR Spectrum of Standard with Sample in 5th Day

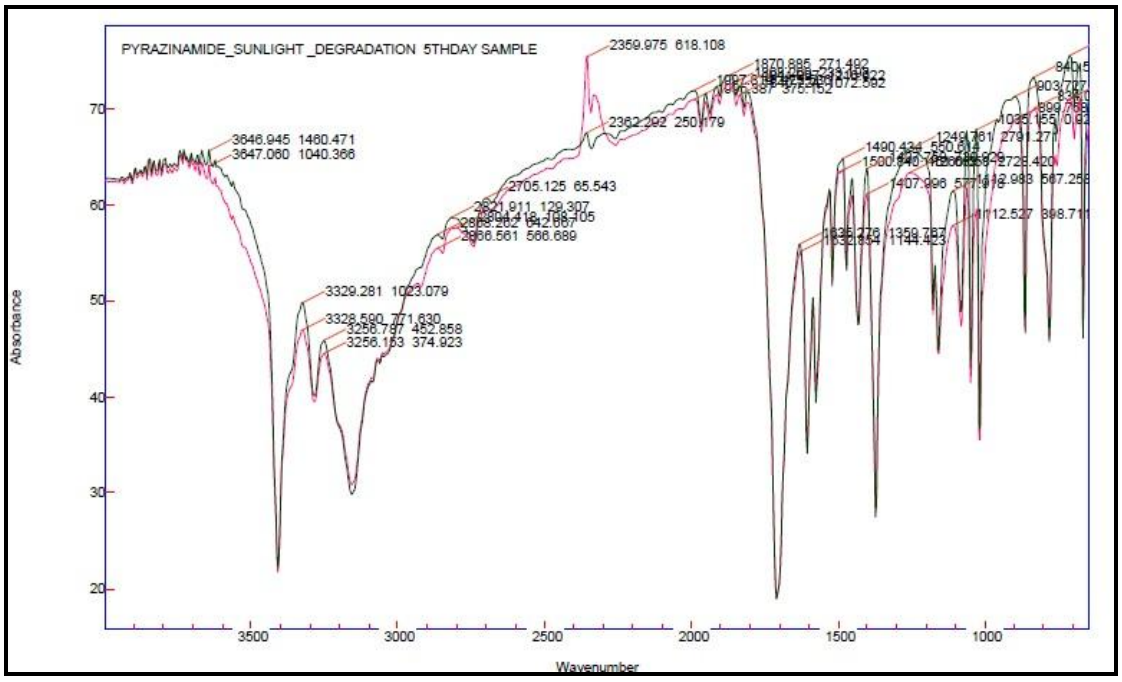


Table: 15 IR study of thermal and photolytic degradation

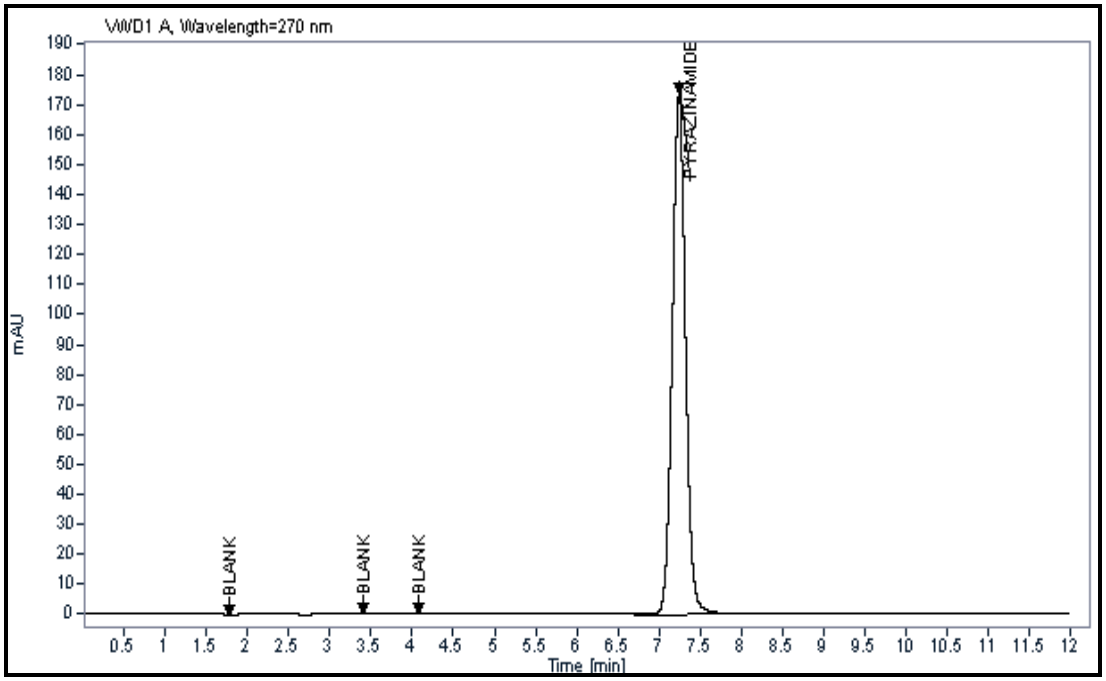
Band Frequency (cm-1)	Bulk	Sample	Result
3412	N-H Stretching	N-H Stretching	No changes observed
3161	C-H Stretching	C-H Stretching	No changes observed
1713	C=O Stretching	C=O Stretching	No changes observed
1610	C=N Stretching	C=N Stretching	No changes observed
1579	C=C Stretching	C=C Stretching	No changes observed

IR Spectroscopy study was performed for interday degradation. The study revealed that there are no changes in the functional group present in the pyrazinamide in bulk and formulations by KBr pellet technique, at the end of 5^{th} day.

UHPLC C-18 silica column was found to be suitable for pyrazinamide .The mobile phase used for assay was acetonitrile and phosphate buffer in the ratio 100:900.The flow rate maintained was1ml/min. The PDA detector was used in the wavelength of 270nm.The retention time was found to be 7 to 7.5.The standard chromatogram is given Fig.35-44 Table-16.

UHPLC STUDY OF BULK AND SAMPLES –UNDER STRESS CONDITIONS

Fig .35-44. STANDARD CHROMATOGRAM



INTRA DAY UHPLC STUDY

The chromatogram for intraday study are given in

Fig.36. Chromatogram of Pyrazinamide Blank in 0.1M NaOH (90mins)

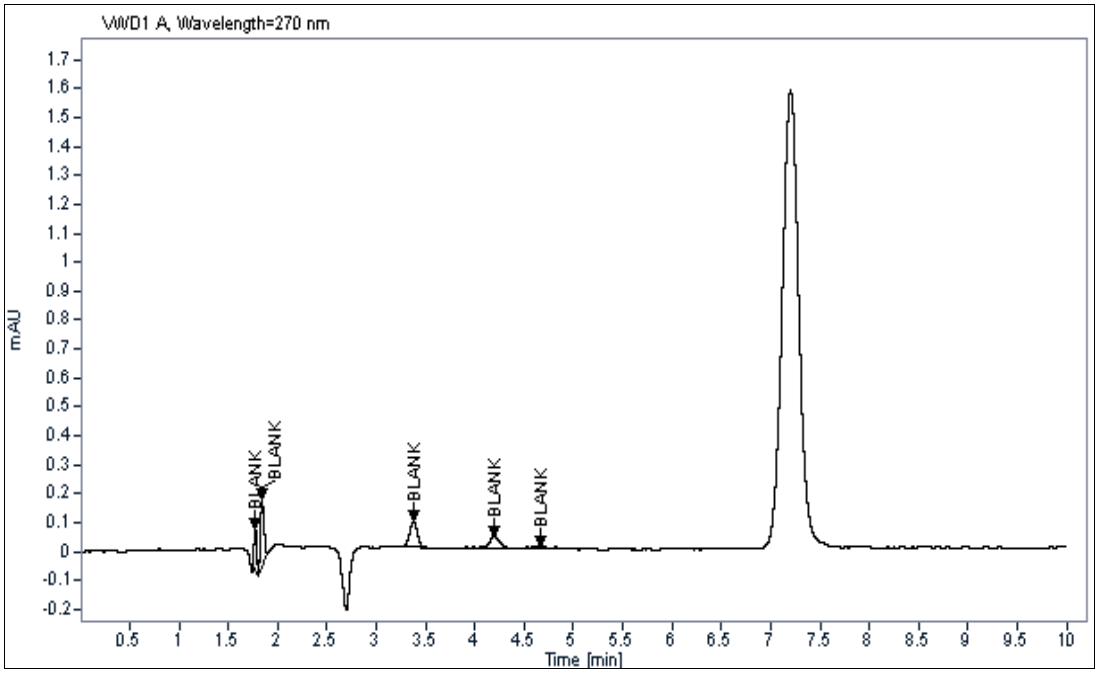


Fig.37. Chromatogram of Pyrazinamide Bulk in 0.1M NaOH (90mins)

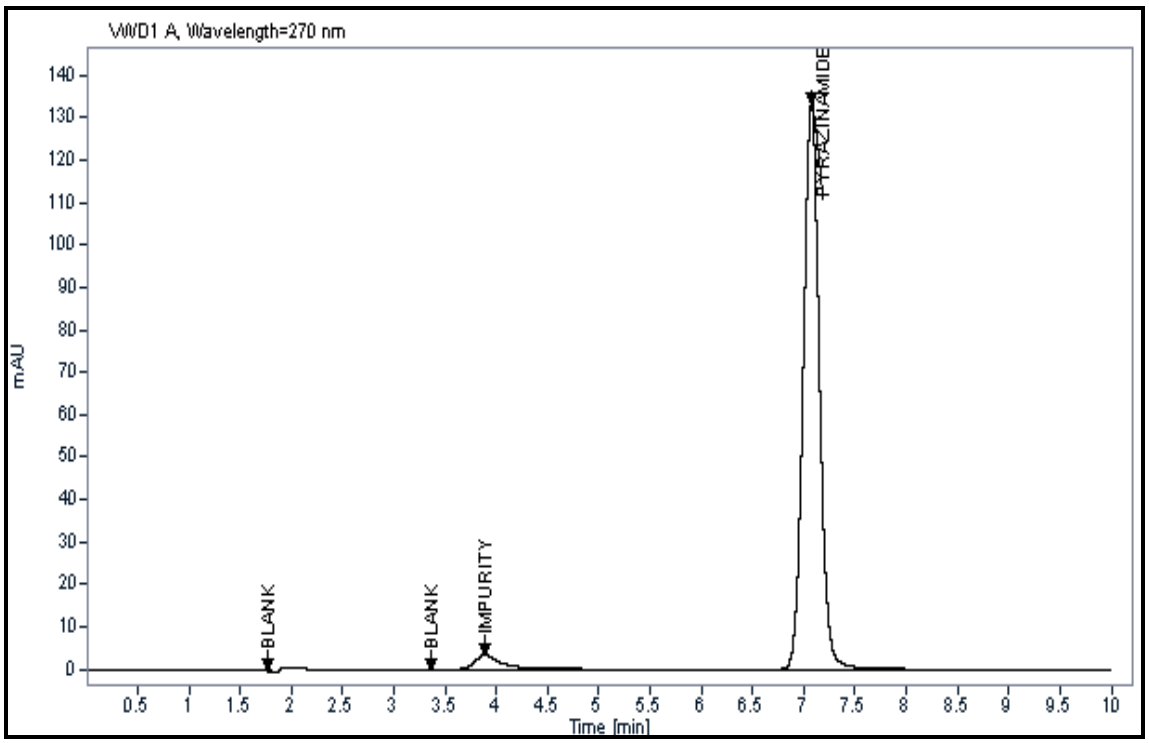


Fig.38. Chromatogram of Pyrazinamide Sample in 0.1M NaOH (90mins)

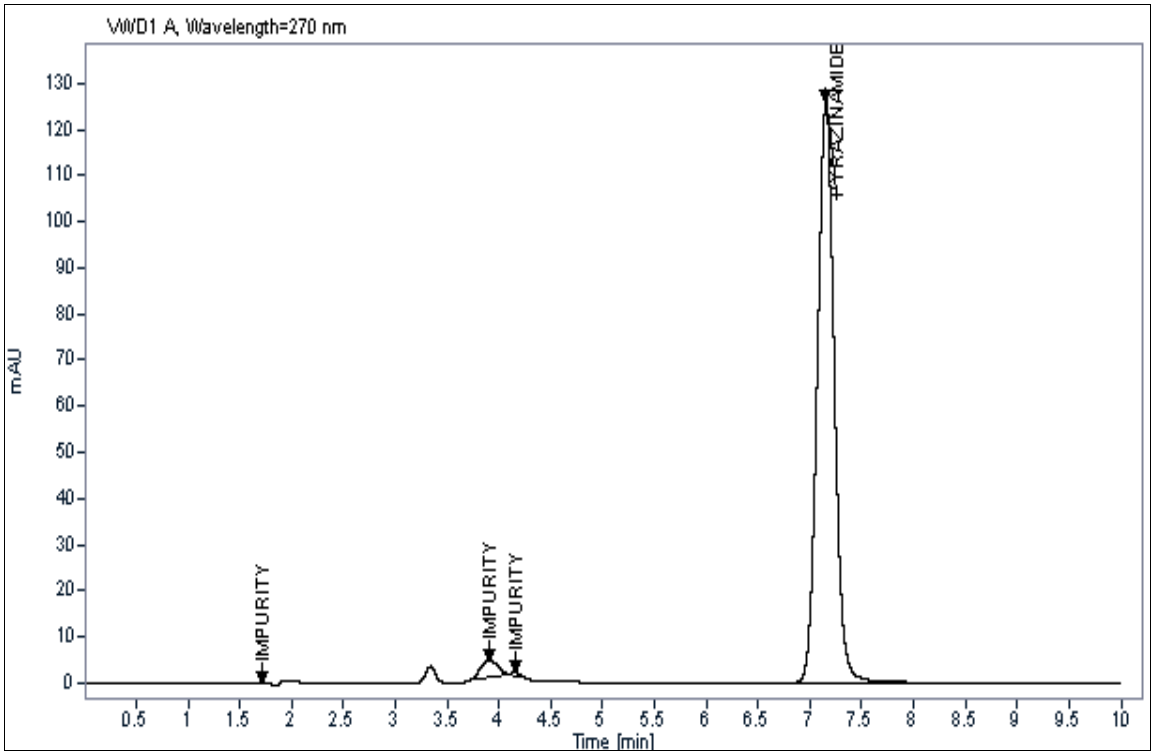


Fig. 39. Chromatogram for Pyrazinamide Blank in 0.1 M HCl (90mins)

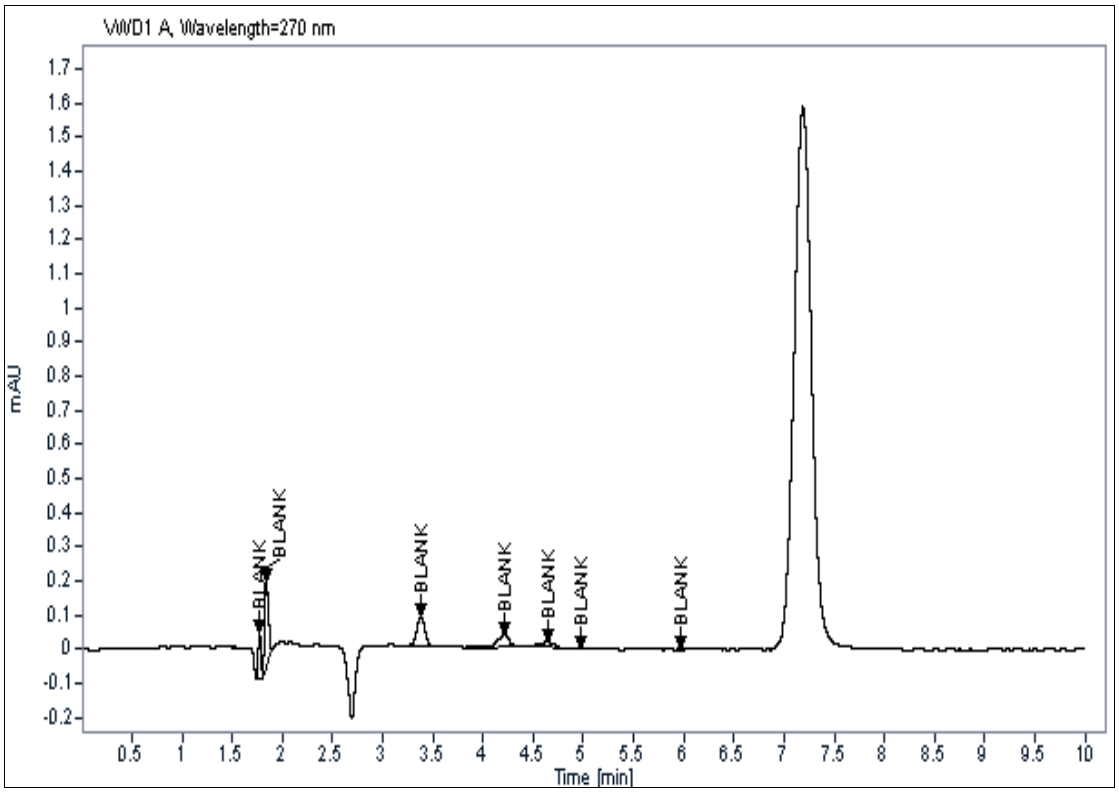


Fig. 40.Chromatogram for Pyrazinamide Bulk in 0.1 M HCl (90mins)

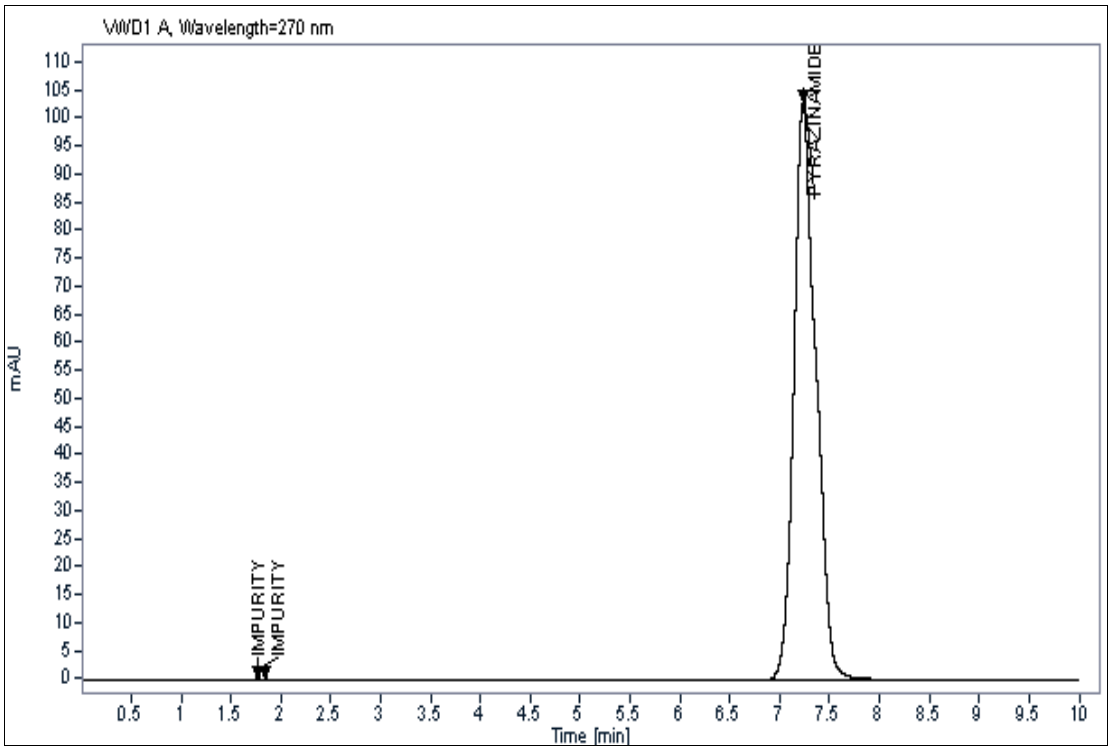


Fig.41. Chromatogram for Pyrazinamide Sample in 0.1 M HCl (90mins)

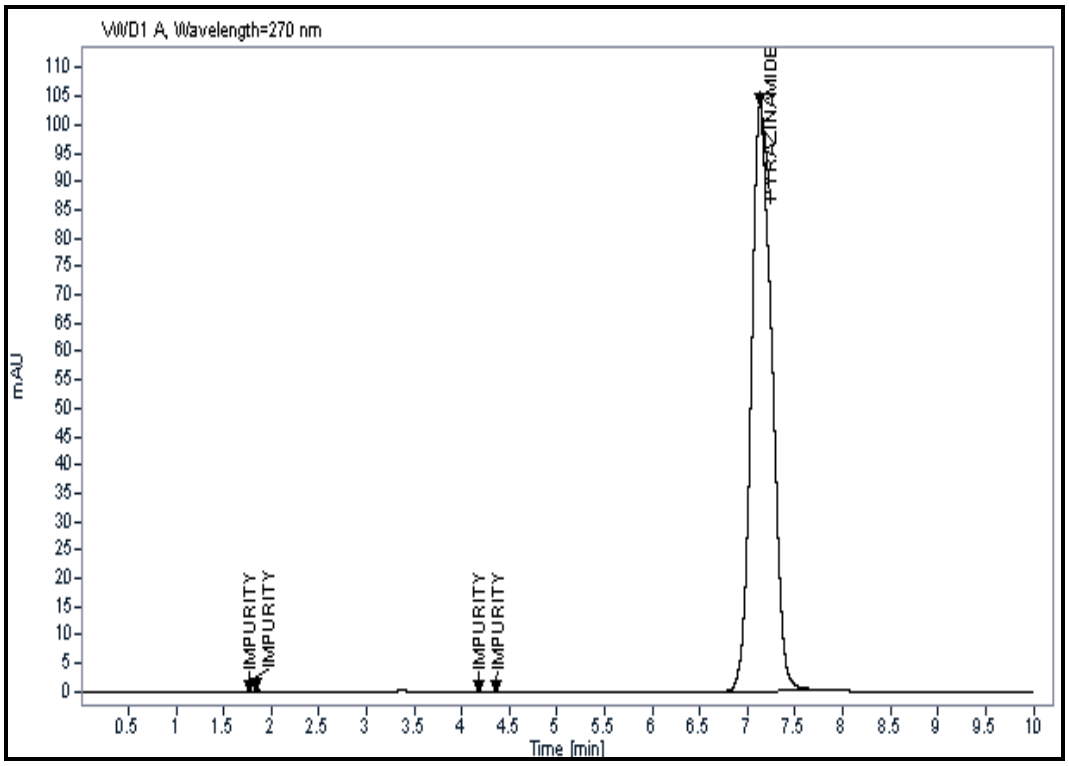


Fig.42.Chromatogram for Pyrazinamide Blank in 5% H_2O_2 (90mins)

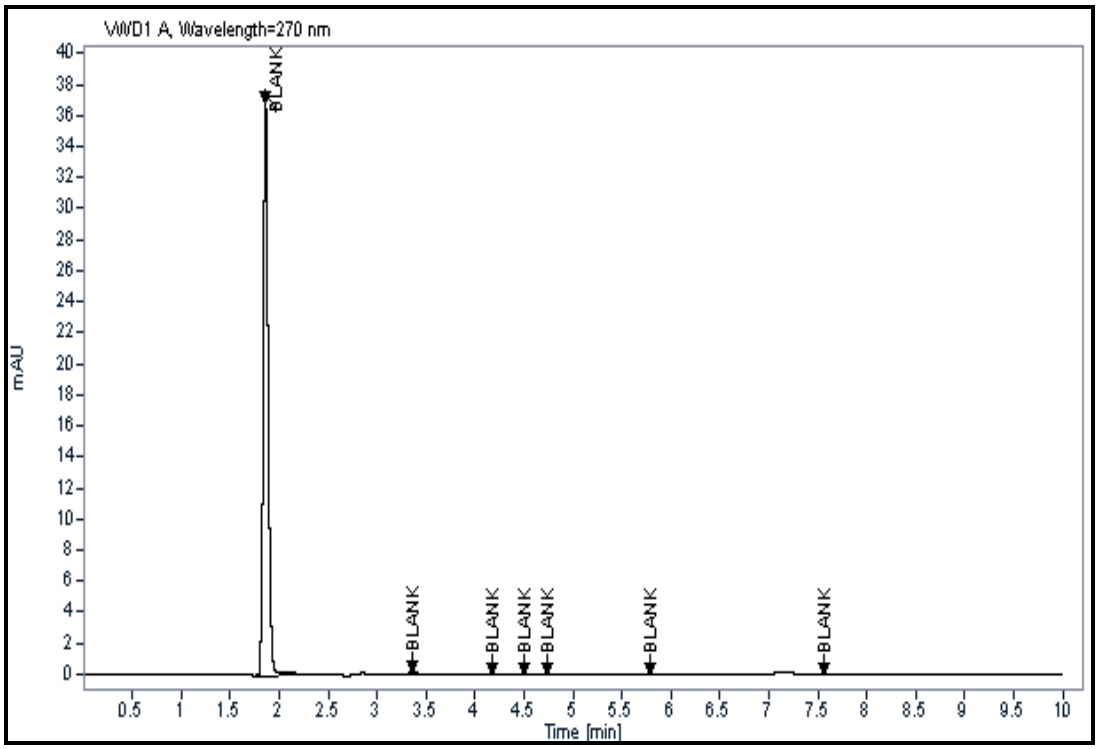


Fig.43.Chromatogram for Pyrazinamide Bulk in 5% H_2O_2 (90mins)

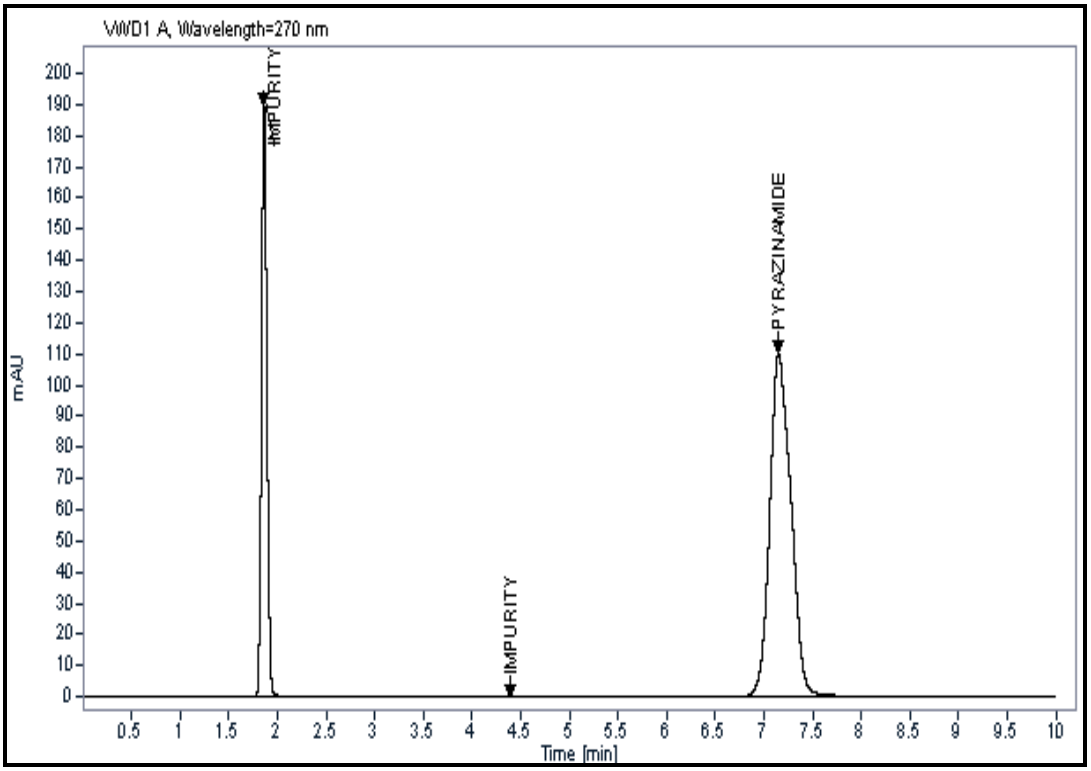


Fig.44. Chromatogram for Pyrazinamide sample in 5% H_2O_2 (90mins)

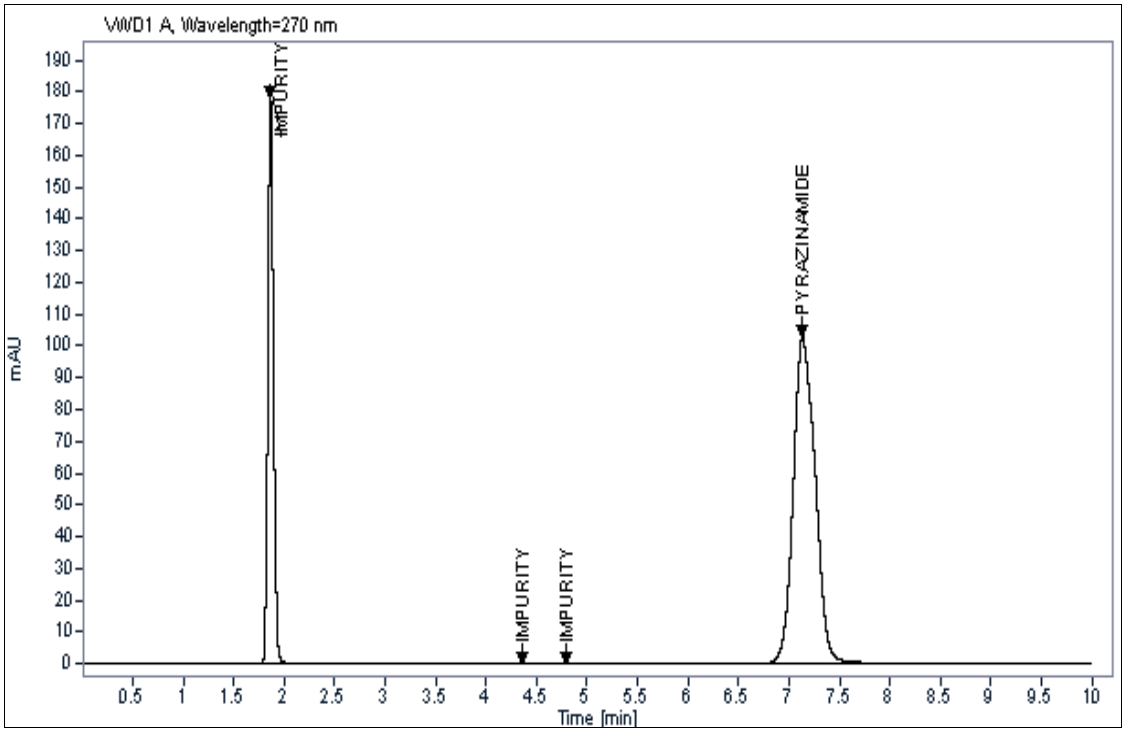


Table-16 Intraday Results for Degradation

Stress Condition	Time	Bulk Peak Area	Sample Peak Area
0.1M NaOH(Alkali)	90mins	1528.40	1481.25
0.1M HCl (Acid)	90mins	1573.21	1551.55
5% H_2O_2(Oxidation)	90mins	2387.02	2245.55

Stress Condition	Time	Bulk Percentage Content (1%)	Sample Percentage Content (1%)
0.1M NaOH(Alkali)	90mins	82.17	79.6
0.1M HCl (Acid)	90mins	84.5	83.4
5% H_2O_2 (Oxidation)	90mins	88.93	83.63

Intraday UHPLC study indicates that pyrazinamide was undergone moderate degradation in hydrolytic and in oxidative study. It was observed that formulation was underwent greater degradation when compared to Bulk.

INTERDAY UHPLC STUDY

Chromatogram for interday study are given in Fig: 45-53, Table-17

Fig: 45.Chromatogram for Pyrazinamide Blank in 0.1M NaOH (3rd day)

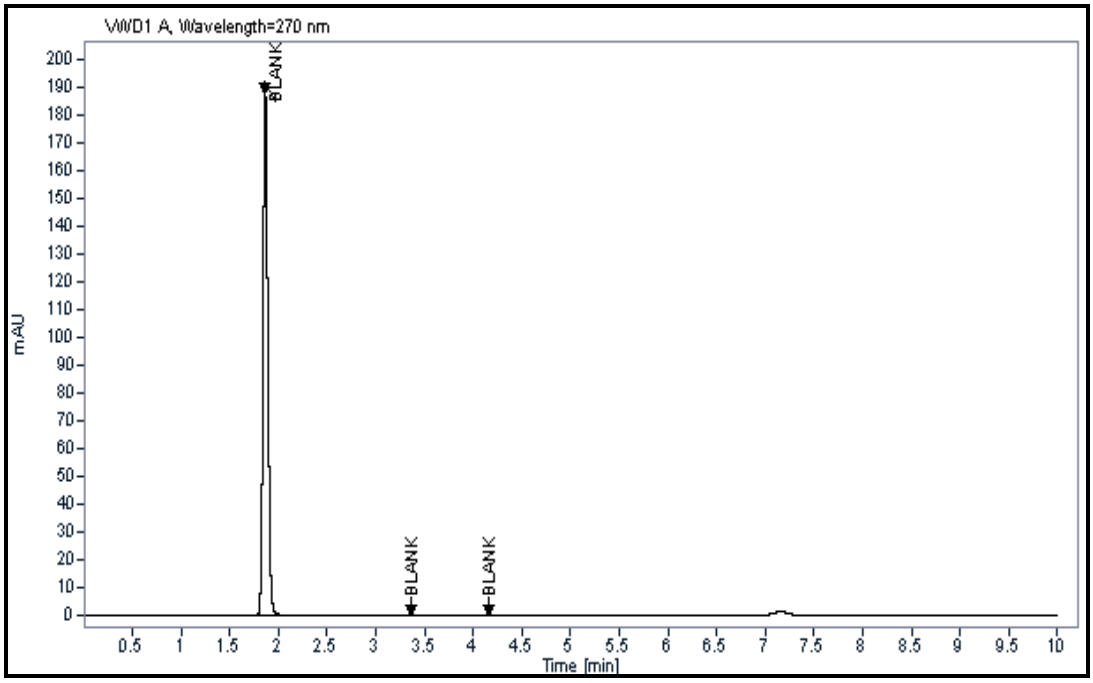


Fig.46. Chromatogram for Pyrazinamide Bulk in 0.1M NaOH (3rd day)

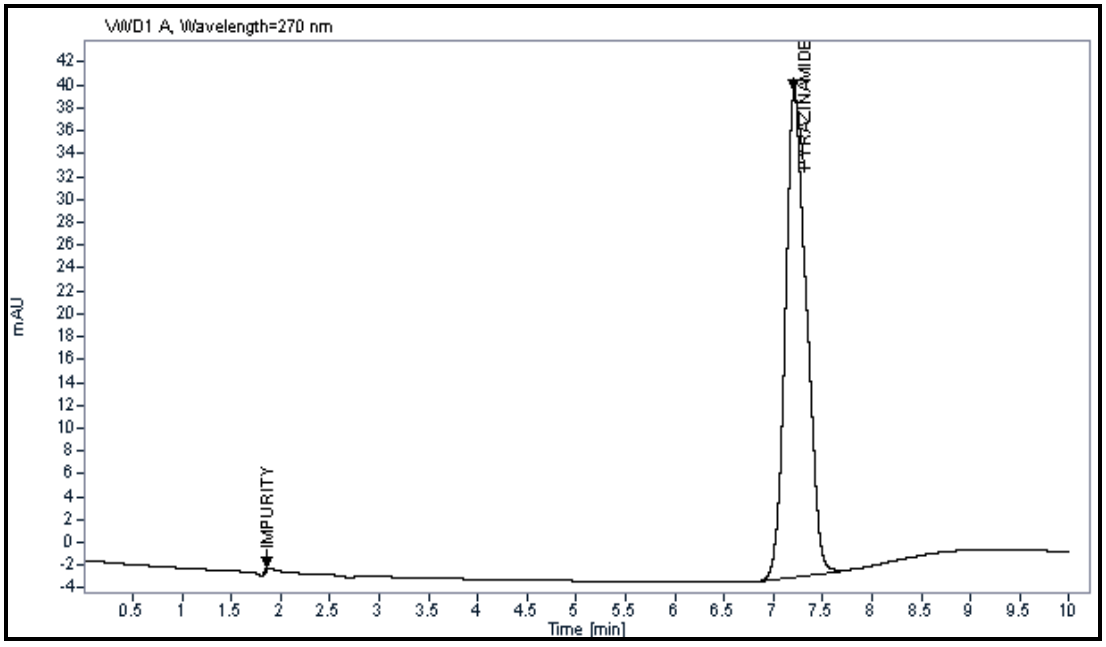


Fig.47. Chromatogram for Pyrazinamide Sample in 0.1M NaOH (3rd day)

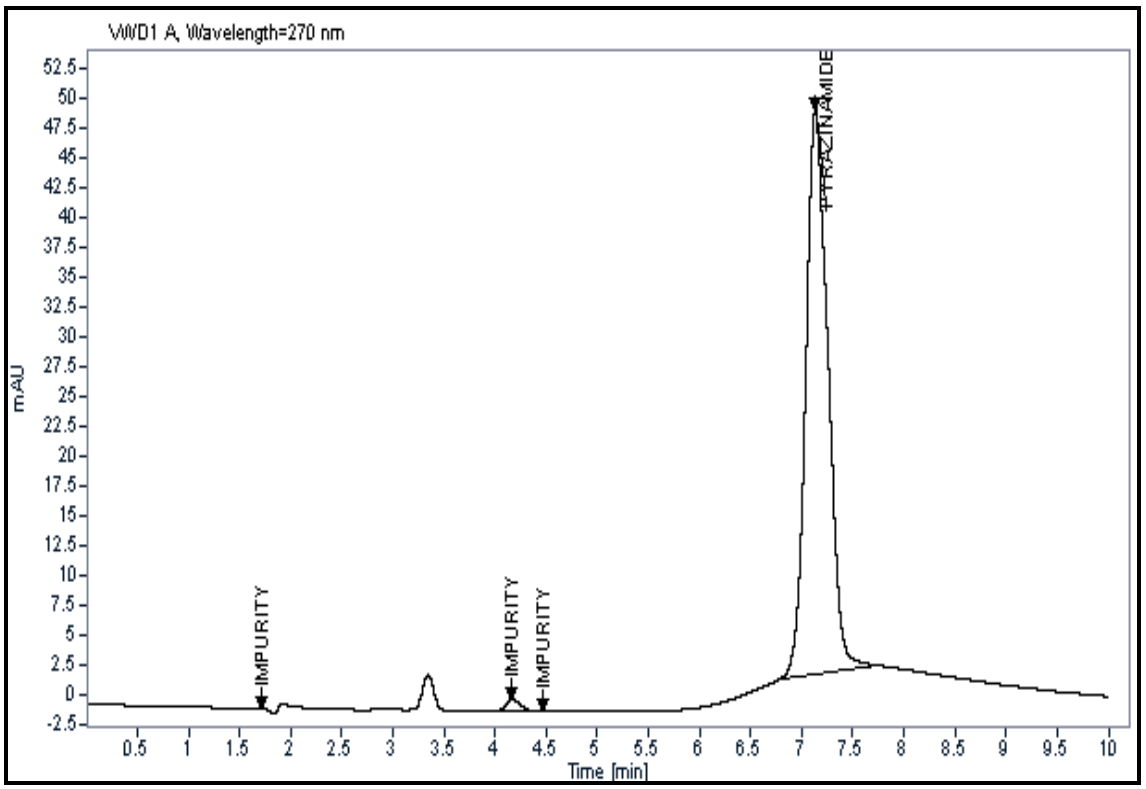


Fig.48. Chromatogram for Pyrazinamide Blank in 0.1M HCl (3rd day)

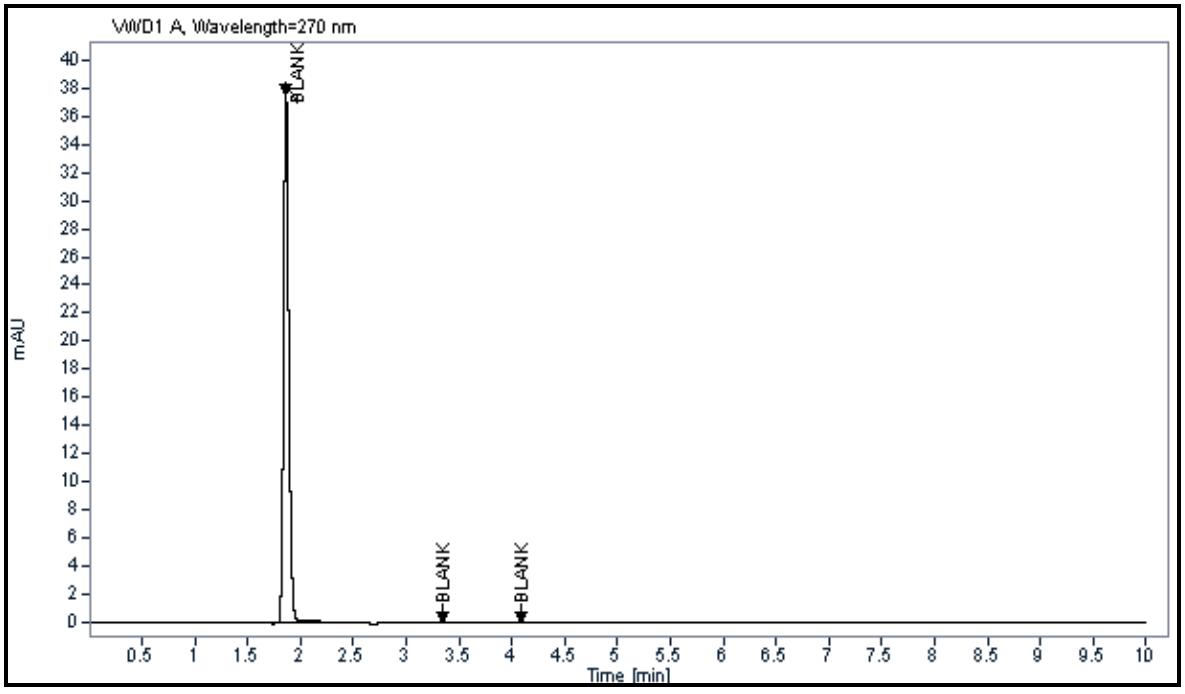


Fig.49. Chromatogram for Pyrazinamide Bulk in 0.1M HCl (3rd day)

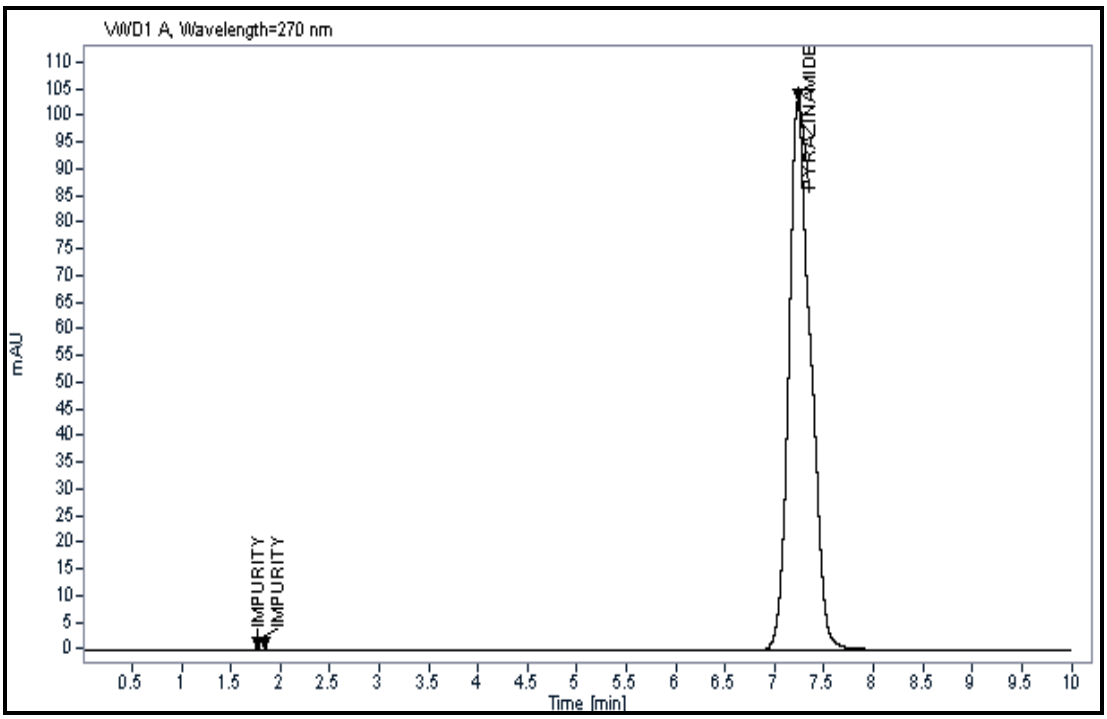


Fig.50. Chromatogram for Pyrazinamide Sample in 0.1M HCl (3rd day)

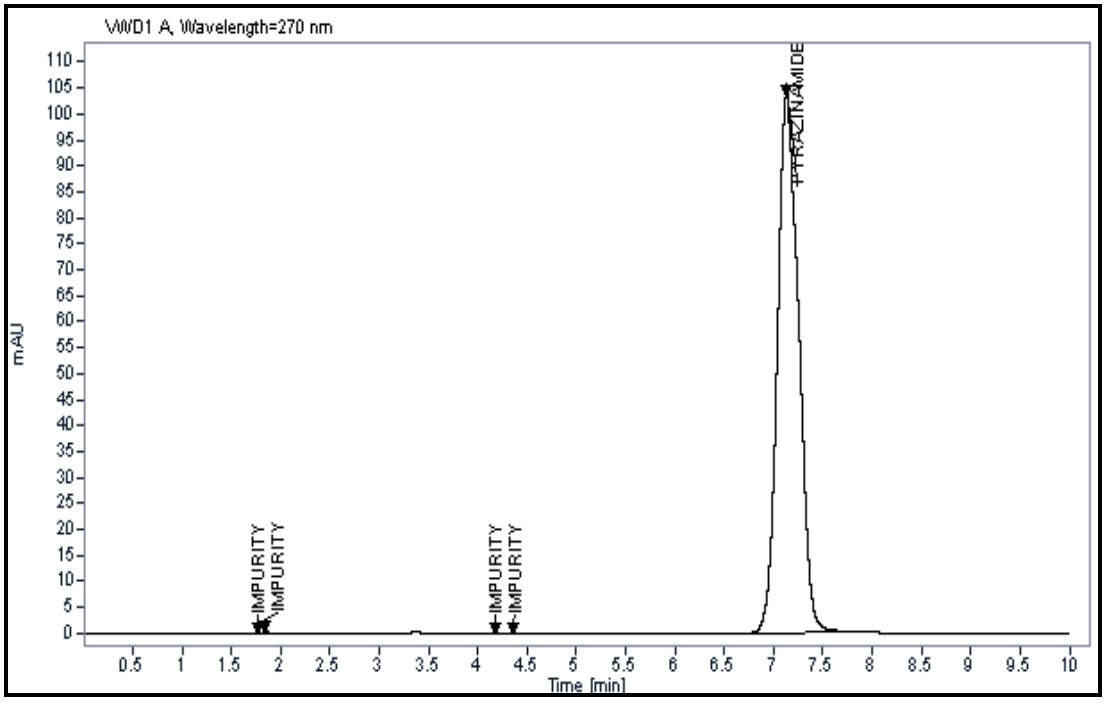


Fig.51. Chromatogram for Pyrazinamide Blank in 5 %H_2O_2 (3^{rd} day)

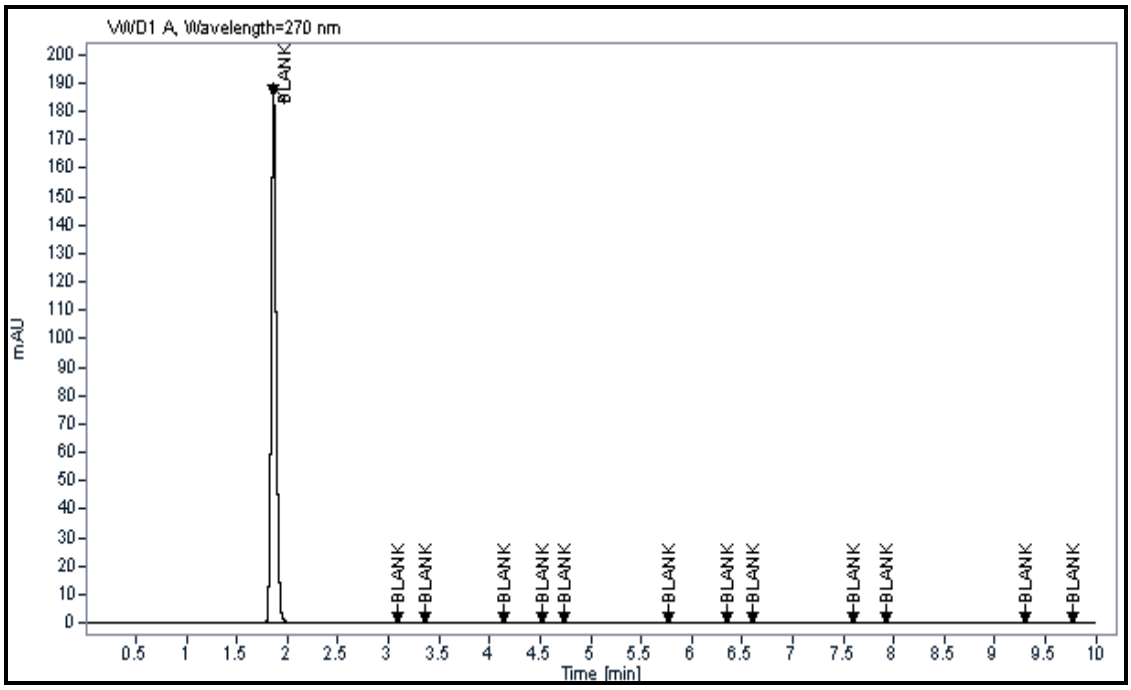


Fig.52. Chromatogram for Pyrazinamide Bulk in 5% H_2O_2 (3^{rd} day)

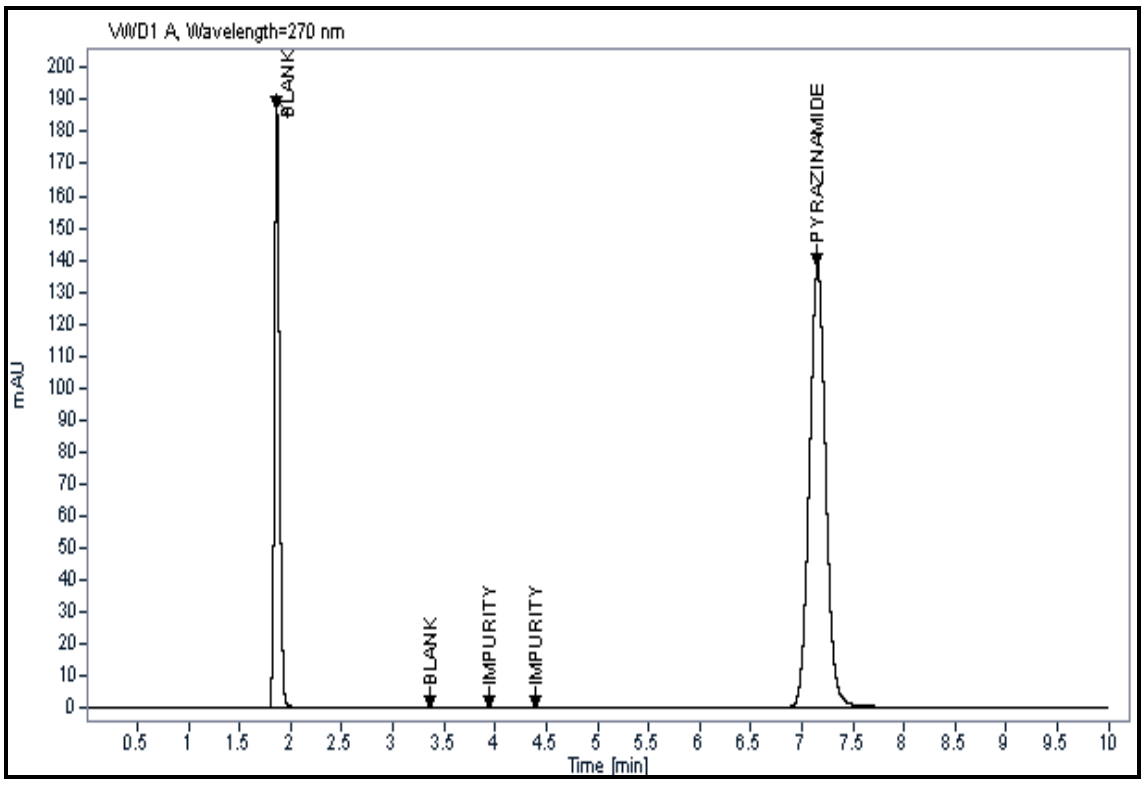


Fig.53. Chromatogram for Pyrazinamide Sample in 5% H_2O_2 (3^{rd} day)

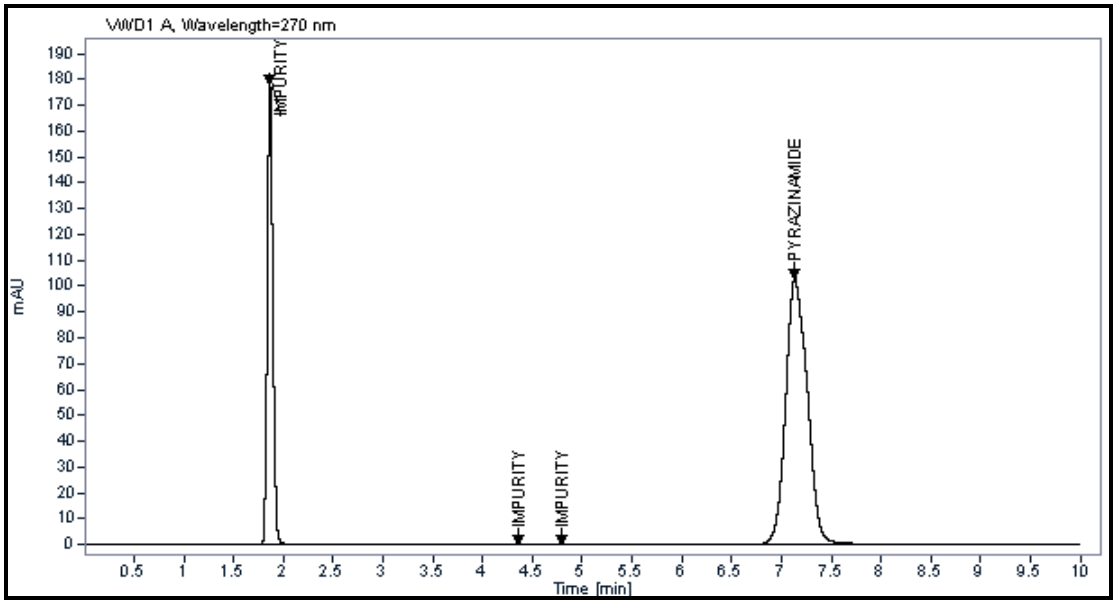


Table-17 Interday Results for Degradation

Stress Condition	Time	Bulk Peak Area	Sample Peak Area
0.1M NaOH(Alkali)	3rd Day	-	-
0.1M HCl (Acid)	3rd Day	-	-
5% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50˚C)	3rd Day	1533.11	1547.31
Sunlight(Photolysis)	3rd Day	1546.71	1480.21

Table- Interday Results for Degradation

Stress Condition	Time	Bulk Percentage Content (1%)	Sample Percentage Content (1%)
0.1M NaOH(Alkali)	3rd Day	-	-
0.1M HCl(Acid)	3rd Day	-	-
5% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50˚C)	3rd Day	82.42	83.18
Sunlight(photolysis)	3rd Day	83.15	79.58

Complete degradation was observed 3^{rd} day hydrolytic and oxidative degradation. This indicates that Pyrazinamide is vulnerable both alkali, acid and 5% H_2O_2. In Photolytic and thermal degradation shows only little amount of degradation.

SUMMMARY AND CONCLUSION

- The present study involves the stress induced stability studies such as alkali and acid hydrolytic degradation, oxidative degradation, thermal and photolytic degradation.
- Degraded samples were quantified by UV and UHPLC method and the results of bulk and samples are compared with that of standard.
- Changes in the functional group in degraded samples were identified by IR. It was found that no major changes in the functional group in the inter day investigation.
- In all the methods used in this degradation study, sample undergoes greater degradation compared with that of standard.
- An important feature in this study was that sample undergoes greater hydrolytic degradation (both acid & alkali) than other degradation methods used. This is because the amide group in pyrazinamide undergoes hydrolysis to form acid. There was only mild degradation in oxidation.

FUTURE SCOPE

- To develop and validate a stability indicating method
- To determine the degradation pathways of drug substance and drug products. (e.g) during development phase.
- To identify impurities related to drug substance or excipients
- To understand the drug molecular chemistry
- To develop more stable formulations.

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